Clostridium difficile in colorectal surgery: a study of local epidemiology, asymptomatic carriage, in-patient disease and surface environmental contamination

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Declaration

The author performed all the investigations and procedures presented in this thesis, unless otherwise stated.

This work has not been submitted for any other degree or professional qualification other then that stated.

Abstract

Clostridium difficile was identified as an infective agent in the late 1970s and early 1980s and causes a spectrum of disease ranging from asymptomatic carriage, mild colitis, pseudomembranous colitis (PMC) to fulminant colitis and even death. Since its recognition as an infective pathogen, *C. difficile* has become the principal cause of nosocomial diarrhoea in adults. The main aims of this four-part thesis were to determine the extent of Clostridium difficile infection (CDI) within the local in-patient population and to establish the epidemiology of CDI within the specialty of colorectal surgery.

The first study focused on the burden of CDI to the diagnostic laboratory and the relative incidence of disease in different clinical specialties over an 8-year period (2000 to 2007) in a region that had not been affected by the hypervirulent 027 strain. A 27-fold increase in the number of faecal samples analysed by the enteric laboratory occurred from 2000 to 2006 and the total number of potential CDI cases increased over the same period, with a decline finally seen in 2007. One-fifth of all toxin-positive samples were from age groups under 60 years of age providing further evidence that CDI was not just a disease of the elderly. Although Medicine of the Elderly provided the greatest faecal analysis workload; Renal Medicine / Transplant Surgery, Intensive Care, Infectious Disease and Gastrointestinal Medicine all had higher incidences of CDI than Medicine of the Elderly. Similarly the low risk group of Paediatrics was also starting to show a small but notable increase in potential incidence. Potential excess costs for CDI in this region rose from £3.5 million to £29 million over the study period.

The second study aimed to assess the potential impact of CDI within all surgical services. In the absence of 027, a further aim of this study was to assess if the more severe and extreme forms of *C. difficile* disease were occurring from 2000 to 2006. Colorectal surgery had the greatest number of CDI episodes followed by Upper Gastrointestinal Surgery and Urology. Despite the total number of *C. difficile* toxin-positive in-patients increasing each year, a similar increase was not demonstrated in the number of patients diagnosed with more severe forms of CDI or in the number of CDI patients treated with surgical intervention. In the cases requiring surgical

intervention up to 40% of patients did not present with diarrhoea and up to 50% of patients did not have a *C. difficile* toxin-positive faecal sample prior to surgery. Demonstrating the importance of clinical recognition of the entire spectrum of *C. difficile* related disease. The post-operative mortality rate for fulminant CDI was 26%. High mortality figures for fulminant CDI treated surgically have not changed significantly over the last two decades and may relate to surgical referral for CDI often occurring late when the patient is in extremis.

The third and fourth studies examined the specific burden of *C. difficile* in the colorectal surgical patient population and the environmental surface contamination within colorectal wards. An asymptomatic carrier rate of 6.1% was identified in the out-patient colorectal surgical population. Asymptomatic carriers admitted from the community play an important role in sustaining the transmission of disease within the hospital environment with 42.8% of *C. difficile* strains only identified in the in-patients faecal samples but not on the surface environment of the wards. Standard enteric hospital laboratory CDI diagnosis using enzyme immuno-assay for toxin A+B detection was 52% less sensitive then toxigenic culture with a false positive rate of 2.5%. Toxigenic culture identified a further 58 colorectal surgical in-patients with CDI. Of all the *C. difficile* isolates identified from in-patients and the surface environment ribotype 001 was the commonest strain, consistent with other local studies where ribotype 001 has emerged as the dominant strain. A large proportion of the in-patient ribotype 001 isolates showed resistance to ceftriaxone and ciprofloxacin. The ribotype 001 isolates from the surface environment showed decreased resistance to ceftriaxone compared with the in-patient strains. Similarly 4.6% of all in-patient isolates showed intermediate resistance to vancomycin but no vancomycin resistance was demonstrated in the environmental surface isolates and may represent increased development of *C. difficile* resistance mechanisms in the host. The patient bed frames were the commonest contaminated environmental surface with *C. difficile*, followed by the patient's bedside lockers and tables. Therefore the risk of a patient ingesting a *C. difficile* spore from the surface environment is high. Following the introduction of a new cleaning protocol during the environmental sampling study a statistically significant reduction in environmental *C. difficile* surface contamination and in the number of CDI colorectal in-patients was demonstrated. Acquisition of CDI from the surface environment in hospitals is not to

be under-estimated and judicious application of infection control measures remains an important factor in preventing CDI transmission.

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Presentations

Clostridium difficile in the paediatric population.
S.N.Reddy, P.Kalima, M.H.S.Collie, D.N.Anderson, I.R.Poxton.
Presented at the European Congress of Clinical Microbiology and Infectious Diseases; Barcelona, 2008.

The Incidence of *Clostridium difficile* in Colorectal Surgery
Reddy S.N., Driscoll P.J., Kalima P., Anderson D.N., Collie, M.H., Mander BJ., Poxton, I. R.
Presented at the American Society of Colon and Rectal Surgeons Triennial Congress; Boston 2008.

Clostridium difficile: changing epidemiology trends 2000-2007
Reddy S.N., Taori S., Kalima P., and Poxton I.R.
Presented at the European Congress of Clinical Microbiology and Infectious Diseases; Helsinki, 2009.

Identification of *Clostridium difficile* in Colorectal Surgery.
Reddy S.N., Fewster G., Mander B.J., Wilson R.G. and Poxton I.R.[1]
Presented at Anaerobe; Philadelphia, 2010.

Clostridium difficile in the Hospital Environment.
Reddy S.N., Fewster J.[2] Mander B.J., Wilson R.G. and Poxton I.R.
Presented at Anaerobe; Philadelphia, 2010.

The following presentations are not presented in this thesis; however they were a continuation of the above work.

Environmental Contamination Of *Clostridium Difficile* In A Radiology Ultrasound Department.

Reddy S.N., Chambers S., and Poxton I.R.

Presented at the Scottish Radiological Society, Glasgow 2011 and

Presented at Anaerobe; San Francisco, 2012

Potential Differences in the Use of Abdominal CT and Imaging Findings between Differing *Clostridium difficile* Strains.

Reddy S.N., Taori S., Ewing F., Brown D., Murchison J.T. and Poxton I.R.

Presented at the European Congress of Radiology; Vienna, 2013

1. Introduction

1.1 Beginnings

Clostridium difficile (C.difficile) is a Gram-positive, anaerobic, motile, spore-forming bacillus. Hall and O'Toole first identified the organism in 1935 from the stool samples of healthy neonates and named it *Bacillus difficilis* due to the difficulties they encountered in isolating the organism. Due to its presence in the faecal samples of healthy neonates it was deemed a commensal, although they demonstrated the organism was highly pathogenic in rabbits and guinea pigs. Finney, a surgeon, first described an entity later recognised as pseudomembranous colitis (PMC), a rarer severe form of disease in 1895, in one of his post-operative patients. Antibiotic-associated colitis and pseudomembranous colitis were later identified in the 1950s; however *Staphylococcus aureus* was thought to be the causative organism (Pearce & Dineen 1960). *C. difficile* was first recognised as an infective agent in 1962 by Smith and King, but they were unable to identify it as the primary causative pathogen of disease.

In 1974 Tedesco *et al.* reviewed 200 patients who had received clindamycin, and found a significant proportion to have antibiotic associated diarrhoea, but that 10% had life-threatening pseudomembranous colitis. However, they were unable to isolate pathogens from the plaque-like lesions (pseudomembranes) seen in the colon. Then in 1977 to 1978 key groups made great strides in the recognition of *C. difficile* as a pathogen. In 1977, Larson *et al.* identified a cytopathic toxin in the faeces of patients with PMC, in the same year Bartlett *et al.* observed typhlitis in hamsters was caused by a clindamycin resistant, toxin producing *Clostridium* species, and Rifkin *et al.* demonstrated *Clostridium sordellii* antitoxin neutralised a clostridial toxin found in the faecal samples of patients with PMC and antibiotic-associated diarrhoea. In 1978, George *et al.* determined that only pure cultures of *C. difficile* caused the same cytotoxic effects as that found in patient faeces neutralised by *C. sordellii* antitoxin. This was also confirmed by Bartlett *et al*, Larson *et al.* and Chang *et al.* in the same year. *C. difficile* is now the most common cause of nosocomial infectious diarrhoea in adults and is considered the primary aetiological agent of pseudomembranous colitis and typhlitis (Bartlett 2002, Khanna *et al.*, 2010).

1.2 The microorganism

1.2.1 Microscopic morphology

C. difficile cells are Gram positive although the stain can be lost in older cultures. They are 2 to 8 μm long with elongated sub-terminal or nearly terminal spores and are motile due to the presence of a few flagella distributed around their surface providing them with characteristic "dancing" motility on wet films.

1.2.2 Macroscopic morphology

C. difficile colonies are flat, white, opaque and non-haemolytic with irregular filamentous edges. On selective media used for *C. difficile* isolation such as cycloserine cefoxitin fructose egg yolk agar (CCFA) and cycloserine cefoxitin egg yolk agar (CCEY) the colonies have a yellowish appearance with greater pronouncement of their filamentous edges. Under UV-light the colonies produce a characteristic chartreuse (yellow-green) fluorescence. *C. difficile* produces a characteristic odour which any health-care worker dealing with patients with *C. difficile* diarrhoea would recognise, it is said to resemble that of a horse stable or dung.

1.3 Virulence factors

1.3.1 The pathogenicity locus (PaLoc)

The PaLoc is a 19.6kb region of the chromosome and is present at the same site, in only one copy in all *C. difficile* strains (Braun *et al.*, 1996). The PaLoc encodes five genes; toxin A (*tcdA*), toxin B (*tcdB*), *tcdC, tcdR* and *tcdE*. Pathogenic *C. difficile* strains produce at least one of the two toxins and in non-toxigenic strains the PaLoc is replaced by a unique conserved 115 bp fragment (Cohen *et al.*, 2000). The PaLoc has feature of a distinct genetic element, with a single integration site, unidirectional orientation and conserved bordering sequences (Braun *et al.*, 1996).

tcdR is the first gene of the PaLoc and is the positive regulator of toxin production. TcdR regulates the transcription of the PaLoc by mediating binding of the RNA polymerase holoenzyme to promoters of the toxin genes (Mani & Dupuy, 2001).

tcdC is the last gene of the PaLoc and is the negative regulator of toxin production. It was found that TcdC destabilises the RNA polymerase-TcdR complex and this prevents it from recognising and binding the *tcdA* and *tcdB* promoters, thereby inhibiting gene expression (Dupuy *et al.*, 2008).

tcdE is located between *tcd*A and *tcd*B and is thought to have a role in the secretion of toxins from within *C. difficile* cells. TcdA and TcdB lack a signal sequence mediating trafficking to the cell membrane (Aktoires, 1997). Secretion of toxins is therefore associated with cell death, however toxin release correlates with sporulation and therefore continued survival of the organism.

1.3.2 Toxin A and toxin B

Toxin A and Toxin B are exotoxins which are taken up from the gut lumen by enterocytes and are the most important virulence factors of *C. difficile*. *C. difficile* exotoxins TcdA and TcdB are large clostridial cytotoxins and have molecular weights of 308kDa and 269kDa respectively (Dove et al., 1990). Conventionally TcdA was defined as an enterotoxin and TcdB as a cytotoxin (Schirmer & Aktoires, 2004). TcdA does have some cytotoxic activity but it is up to a 1000 times less potent than that of TcdB. TcdB was initially believed to have no enterotoxic activity (Sullivan *et al.*, 1982) however TcdB has now been observed to be as enterotoxic as TcdA (Savidge *et al.*, 2003) and a study by Lyras *et al.*, (2009) provides evidence that of the two toxin B is more important.

Toxins A and B have three regions; the N-terminal catalytic domain, the C-terminal toxin-receptor binding domain and a putative translocation domain (von Eichel-Streiber *et al.*, 1992). This three-part toxin structural model has now been replaced with a four domain structural model which involves the glucosyltransferase domain, the cysteine protease domain, the translocation domain and the receptor-binding domain (Albessa-Jove *et al.*, 2010).

The N-terminal is responsible for the catalytic activity of the toxins, the C-terminal mediates cell-binding (Sauerborn *et al.*, 1997), the cysteine region plays a role in

endocytosis (Barroso *et al.*, 1994) and the translocation domain as the name suggests is essential for translocation (von Eichel-Streiber & Sauerborn, 1990).

Until recently it was thought that both toxins A and B were required to elicit disease however *Clostridium difficile* infection (CDI) outbreaks have been caused by A-B+ strains, which refutes this view (Kuijper *et al.*, 2001).

Cell-binding

The interaction of toxin receptors is the primary process of entry of toxins into cells. The C-terminal binding domain is composed of repetitive oligopeptide elements known as CROPs and are considered the host cell surface polysaccharide binding sites (Just & Gerhard, 2004). Carbohydrate receptors for TcdA were identified in the brush border membranes of hamsters and TcdA has been shown to bind on human cells to a disaccharide present on the I, X and Y antigens (Tucker & Wilkins, 1991). TcdB has been found to bind to a variety of cell types however its receptor has not been identified (Voth & Ballard, 2005). The toxin and receptor interaction results in receptor-mediated endocytosis (Just & Gerhard, 2004). TcdA and TcdB are then processed to reach the cytosol where they are able to induce cytotoxic effects.

Membrane translocation

Translocation of the toxins into the cytosol requires acidification of an endosome. In response to an acidic pH in the endosome the toxins undergo structural changes that expose hydrophobic domains and result in the insertion of the toxin into the endosomal membrane (Qa'Dan *et al.*, 2000). Barth *et al.*, (2001) found that cytotoxic activity of toxin B could be inhibited by treating the cells with a proton pump inhibitor. Pfeifer *et al.*, (2003) demonstrated that the C-terminal of toxin B remained in the endosome whilst the N-terminal was found in the cytosol. TcdB undergoes proteolytic cleavage by a cysteine which is activated by inositol hexakiphosphate (Egerer *et al.*, 2009).

Activity of Enzymes and the effects of glucosylation on GTPases

Cytosolic targets of TcdA and TcdB are proteins belonging to the *Rho* and *Ras* families. These proteins are GTPases and belong to a superfamily of low molecular weight proteins, which act as molecular switches in a variety of key-signalling

pathways. These include adhesion, epithelial barrier function, immune cell-migration, progression of the cell-cycle, secretion of cytokines, phagocytosis and endocytosis (Aktoires *et al.*, 2000).

The GTPases alternate between active and inactive forms; an inactive GTP-bound form in the cytosol and an active GTP-bound form at the cell membrane. In the cytosol nucleotide exchange is prevented by Rho proteins binding to the nucleotide dissociation inhibitor. Following receptor-mediated interaction the GTPases translocate to the cell membrane and here in a process catalysed by guanine exchange factors GDP is exchanged for GTP. GTP is then hydrolysed to GDP by GTPase activating proteins which return GTP to its original form. Both TcdA and TcdB glucosylate the target protein resulting in the inactivation of the GTPases (Schirmer & Aktoires, 2004).

The glycyosylation of Rho proteins and subsequent lethal depolymerisation of the actin cytoskeleton in epithelial cells leads to cell rounding and detachment form the basal membrane (Mahida *et al.*, 1996). Inactivation of the the GTPases results in the breakdown of the tight junctions of the colonic epithelial barriers. This increases the permeability of the epithelial barrier resulting in diarrhoea. The resultant acute inflammatory response stimulates the release of pro-inflammatory cytokines and allows neutrophils, mast cells and macrophages to pass through the tight junctions (Kelly & Kyne., 2011). TcdA also induces enteric neurones to secrete substance P which causes mast cell activation further increasing mucosal secretion and inflammation (Castagliuolo *et al.*, 1997). The presence of neutrophils, mucus, fibrin and sloughed epithelium within the gut lumen results in the formation of pseudomembranes (Linevsky *et al.*, 1997), as the inflammatory response continues toxic megacolon and perforation can ensue.

1.3.3 Binary toxin

Some *C. difficile* strains produce a third toxin known as binary toxin (CDT). The binary toxin consists of two molecules (subunits) which are both required for activity (Rupnick *et al.*, 2003). The subunit CDTa is the enzymatic subunit which causes cytoskeletal disruption and subunit CTDb is the binding subunit which allows translocation of the enzymatic subunit CDTa into the cytoplasm (Barth *et al.*, 2004).

This toxin has similar characteristics to the binary toxins in other clostridia and does not produce the cytopathic effect usually seen with TcdA or TcdB.

The role of the binary toxin in CDI pathogenesis is not wholly understood and it is thought to contribute to severity rather than act as a key component in CDI development as A-B+ CDT+ strains in a hamster model produced colonisation but no disease (Geric *et al.*, 2006). The prevalence of binary toxin genes was reported in only 17.2% of toxigenic strains in a European study (Barbut *et al.*, 2007), but those strains with binary toxin caused more severe disease and increased mortality.

1.3.4 Surface-associated proteins

To establish infection the bacteria must be able to bind to host cells, colonise tissues, invade them and continually interact and modulate the host immune system to allow disease to persist (Pizarro-Cerda & Cossart, 2006). Therefore adherence of *C. difficile* to the gut mucosa is vital to sustain CDI and this is achieved with a number of cell-surface proteins known as adhesions.

The S-layer protein is a protective surface comprised of two sub-units. These S-layer proteins are involved in adherence to mucus and epithelial cells (Calabi *et al.*, 2002) and were found to be the most commonly recognised antigens in CDI patients (Wright *et al.*, 2008). The flagella of *C. difficile* may also be involved in adherence mechanisms. The flagellar filament (FliC) and flagellar cap (FliD) can both mediate mucus binding an important first step in colonisation (Tasteyre et al., 2001) and antibody responses to both these antigens in patients have been observed (Pechine *et al.*, 2005).

Another surface protein Fbp68 is a fibro-nectin binding protein present mainly in the cytoplasmic membrane of *C. difficile* cells and has been found to induce a high antibody response in patients (Hennequin *et al.*, 2003, Pechine *et al.*, 2005). Cwp84 and CwpV are further surface-associated proteins and important virulence factors.

1.3.5 *Clostridium difficile* spores

C. difficile produces spores that are metabolically dormant. These spores are important in the pathogenesis, persistence and transmission of CDI. *C. difficile* spores

can persist in the environment for long periods of time and are resistant to environmental changes including disinfection as discussed further in Chapter 6.

These spores once ingested attach to the gastrointestinal tract via proteinaceous exosporial filaments (Panessa-Warren *et al.*, 1997). Only vegetative cells are able to produce spores and therefore germination and outgrowth of the spores is vital to CDI. Spore germination is mediated by receptors once the spore is in a nutrient-rich environment. The spores have been found to germinate in response to bile salts with germination in humans stimulated by taurocholate in the small intestine (Howerton *et al.*, 2011).

C. difficile are unable to undergo germination and outgrowth in the presence of normal gut flora. Normal gut flora is able to modify primary bile salts to secondary bile salts. The primary bile salt chenodeoxycholate can inhibit spore germination due to the decreased affinity of spores to taurocholate in its presence. When the gut is stripped of its normal intestinal flora, following exposure to certain risk factors such as antibiotics, the ratio of primary to secondary bile salts changes, this allows spores to germinate into vegetative cells which can produce toxins (Giel *et al.*, 2010). The precise germination factor however has not been identified.

1.4 Typing methods

Typing of *C. difficile* isolates is vital to the comprehension of its epidemiology and its ability to cause disease. Initially techniques employed methods to identify phenotypic characteristics of *C. difficile* whereas current methods assess the genotype.

1.4.1 Phenotypic methods

The initial methods used resistance patterns to antibiotics to type strains (Burdon, 1982), crossed immunoelectrophoresis, analysis of plasmid profiles, analysis of soluble proteins and protein patterns by SDS-PAGE, and bacteriocin and bacteriophage typing methods rapidly followed (Wust et al., 1982; Poxton *et al.*, 1984) and proved to be useful. The immunochemical finger-printing of EDTA-extracted cell surface proteins was also used in studying out-breaks of CDI (Poxton *et al.*, 1984).

Serogrouping was developed by Delmee *et al.*, (1985 and 1986) who were able to identify 19 different sero-groups and a comparison of serogrouping with SDS-PAGE protein profiles showed that either method could be used to type *C. difficile* isolates. Serogrouping is often used as a comparative standard with other schemes (Brazier, 2001).

Surface-layer typing (S-layer typing) was a further phenotypic method of typing *C. difficile*. This method interrogated the inter-strain variability in the molecular mass of the high and low weight surface layer proteins (McCoubrey & Poxton, 2001; McCoubrey *et al.*, 2003).

1.4.2 Restriction enzyme analysis

This method of *C. difficile* typing uses restriction endonuclease analysis (REA). Kuijper et al., (1987) used *Hind*III, a frequent cutter, to restrict whole cell-DNA which produced distinguishing restriction patterns for different *C. difficile* strains on agarose gels, these were compared by eye to existing exemplars and the method correlated well with the strains' protein profiles. The method was reproducible and highly discriminatory but technically demanding.

1.4.3 Pulse field gel electrophoresis

Pulse field gel electrophoresis (PFGE) is a typing method that analyses the whole genome of an organism following restriction using rare cutting enzymes such as *Sma*I and *Sac*II (Brazier 2001). This method was also highly discriminatory and correlated well with serogrouping. PFGE is commonly used in North America and the strains are assigned NAP (North American pulsed-field) types.

1.4.4 Ribotyping

This method of ribotyping is based on the variability in length of the 16s – 23S spacer regions present in different alleles. It was first suggested as a typing method in 1993 (Gurtler, 1993). It had been reported that *C. difficile* carried 14 copies of the rRNA gene each with intergenic spacer regions of varying lengths. By amplifying these regions of DNA and running them on long denaturing polyacrylamide gels Gurtler identified different banding patterns for different strains. Cartwright *et al.* (1995)

found the method to be simple and reproducible allowing them to differentiate a large number of isolates. The method was modified by O'Neill *et al.* (1996) and became the typing method of choice by UK reference laboratories with 116 ribotypes defined in 1996 (Stubbs *et al.*, 1999). They had modified the method by extracting DNA by boiling with resin and re-designed the primers to give bands between 260 and 585 bp and used high resolution agarose. Recently capillary gel electrophoresis-based PCR ribotyping has been developed, which seems to overcome the problems of inter-laboratory variation (Indra *et al.*, 2008).

1.4.5 Toxinotyping

Toxinotyping uses the inter-strain variability in the toxin genes *tcd*A and *tcd*B (Rupnik et al., 1997) which encode for toxins A and B. The variations are concentrated in the A3 fragment and B1 fragment. The restriction of these DNA regions with the enzymes *Eco*RI, *Acc*I and *Hinc*II and the resultant length of the products is then analysed with electrophoresis. This method has correlated well with PFGE, serogrouping and ribotyping however ribotyping remains more discriminatory. Strains which carry a PaLoc identical to that of the reference strain VPI10463 are known as toxinotype 0; currently there are 24 variant toxinotypes.

1.4.6 MLVA and MLST

Multilocus sequence typing (MLST) and multilocus variable-number tandem-repeat analysis (MLVA) are useful in determining epidemiological links between isolates and therefore can help differentiate between isolates of the same serogroups, toxinotypes and ribotypes and their subtypes and is therefore very useful in CDI outbreak situations. With MLST by sequencing the toxin genes and their positive regulator, the binary toxin gene and other virulence involved genes, several *C. difficile* clones can be identified (Lemee *et al.*, 2005). MLVA is a technique that amplifies seven regions featuring short tandem repeats distributed over the genome, different isolates are distinguished by analysis of repeat numbers (van den Berg *et al.*, 2007).

1.4.7 *slpA*ST

The *slpA* gene encodes the precursor of the S-layer proteins. The inter-strain differences in the variable region of the *slpA* gene of *C. difficile* were sufficiently

discriminatory to be used as a typing scheme (Karjalainen *et al.*, 2001). Variable regions of the gene are amplified by PCR and analysed by restriction digest and DNA sequencing and can be used to discriminate between types and subtypes.

1.5 *Clostridium difficile* out with the human host

C. difficile is widely distributed in the environment. An extensive survey performed in Wales found *C. difficile* in many water sources including the sea, rivers, lakes, tap water and swimming pools and also in soil samples and on root vegetables (al-Saif & Brazier, 1996). Bakri *et al.*, (2009) isolated *C. difficile* from 7.5% of packaged supermarket salads. Metcalf *et al.*, (2010) found *C. difficile* in 4.5% of vegetables purchased from a greengrocer.

Animals are a further potential source of *C. difficile* to humans. CDI colitis has been diagnosed in several neonatal pigs (Songer, 2004) and carriage has been reported in horses, calves and domestic pets (Arroyo *et al.*, 2007; Rodriguez-Palacios *et al.*, 2006). The predominant toxinotype in pigs and calves is toxinotype V which is now being seen more in human CDI cases across the globe. Ribotype 078 toxinotype V isolates from diarrhoeal pigs and human patients in the Netherlands were found to be both phenotypically and genotypically identical (Debast *et al.*, (2009). This suggests commonality between animal and human strains or the possibility of zoonotic transmission.

C. difficile has also been isolated from a variety of meat products. Songer et al., (2009) found heavy contamination of up to 40% of the raw meat products they tested sold in North America and Weese *et al.*, (2009) found 71% of ground meat products to be contaminated with *C. difficile* and in 2010 they found *C. difficile* in 12.8% of retail sold chicken. Much lower *C. difficile* rates were identified in meats sold in Europe, Bouttier *et al.*, (2010) found *C. difficile* in only 1.9% of tested products and this 1.9% was found only in vacuum-packed meats. A study performed in our laboratory did not identify any *C. difficile* from the 100 packaged meats tested (Jobling & Poxton unpublished). The higher incidence found in meats that are packaged suggest contamination during the processing (Songer *et al.*, 2009).

The significance of the presence of *C. difficile* and its ability to transmit disease to human hosts from food and other environmental sources mentioned is uncertain.

1.6 *Clostridium difficile* disease acquisition and risk factors

1.6.1 Acquisition of *C. difficile*

Clostridium difficile is spread by the faecal-oral route and ingestion of C. *difficile* spores into the gastrointestinal tract is the first step towards development of CDI. Contaminated hands are the most common method for ingestion by the host. *C. difficile* is able to colonise the host and under the correct environmental conditions within the gut the spores are able to germinate into vegetative cells which produce toxins and other virulence factors. These trigger the host's inflammatory response within the gastrointestinal tract resulting in symptomatic disease. The host also goes on to produce and disseminate more spores.

Symptomatic CDI patients are the main source of disease transmission. These infected patients shed large numbers of vegetative cells and spores into the surrounding environment (Wilcox *et al.*, 2003). This is particularly significant in nosocomial settings where the close proximity of individuals allows spores to spread and cross-contaminate other patients via commonly touched surfaces.

1.6.2 Risk factors

Antibiotics

The disruption of the indigenous gut flora means that it is no longer able to provide protection against enteric pathogens. Antibiotics are the most common causal agent that disrupts the normal gut flora resulting in symptomatic CDI. Many early experiments used clindamycin as this was the antibiotic CDI was initially synonymous with and its effects were demonstrated in animal models (Tedesco *et al.*, 1974; Chang *et al.*, 1978). The replacement of clindamycin with broad-spectrum penicillins and third-generation cephalosporins led to their use becoming the most implicated cause of CDI (Bartlett, 2008). Starr *et al.*, (2003) found that the use of third-generation cephalosporins increased the risk of an individual becoming culture positive and also demonstrated that an individual's risk of becoming toxin-positive increased with antibiotic use. More recently fluoroquinolones have become implicated

in CDI development as their wide-spread use has increased so has *C. difficile* resistance to fluoroquinolones (McDonald *et al.*, 2005; Mutlu *et al.*, 2007). Newer fluoroquinolones have greater activity against anaerobes and are therefore more likely to disrupt the host's normal gut flora; they may then exert selective pressure allowing fluoroquinolone resistant strains to emerge during fluoroquinolone treatment (Guerding 2004). Deshpande *et al.*, (2008) suggested an association between fluoroquinolone use and CDI caused by the hypervirulent 027 ribotype and this was later supported by Sundram *et al.*, (2009). The duration of antibiotic exposure and the number of antibiotics a patient is treated with are further risk factors for CDI development (Pepin *et al.*, 2005).

Age

Nosocomial CDI is primarily seen in those over 65 years (Sunenshine & McDonald, 2006; McFarland *et al.*, 1995). This population's susceptibility to CDI is thought to relate to increased co-morbidity such as underlying disease including chronic renal impairment and malignancy; increased exposure to common risk factors such as antibiotics and increased hospitalisation. These patients are also more likely to be on immunosuppressive agents decreasing their immune response to enteric pathogens and have greater alterations in the normal gut flora (Hopkins *et al.*, 2002).

CDI is now also being diagnosed in previously low risk groups mainly children peripartum women and young healthy adults (Benson *et al.*, 2007; Kim *et al.*, 2008; Rouphael *et al.*, 2008). Most of these infections are thought to be community acquired and are less likely to be associated with morbidity or mortality (Naggie *et al.*, 2010). However mortality has been associated with the peripartum group in particular (Rouphael *et al.*, 2008).

Co-morbidity

Co-morbidity and immunosuppression increases with age along with the associated risk of CDI (Pepin *et al.*, 2004). Co-morbidities include diabetes mellitus, HIV infection, malignancy, cellulitis, anaemia, burns patients, and gastro-intestinal, pulmonary and urinary tract infections to name but a few (Kyne *et al.*, 2002, Changela *et al.*, 2004; Polgreen *et al.*, 2010).

Inflammatory bowel diseases including Crohn's disease and ulcerative colitis have both been associated with higher rates of CDI (Riccardi *et al.*, 2009; Nguyen *et al.*, 2008). These patients are often from a younger patient cohort. Increased mortality has been reported in patients with inflammatory bowel disease and CDI then inflammatory bowel disease alone (Pituch, 2009).

Patients who have undergone recent surgery are also at increased risk of CDI development with CDI rates in patients who have undergone abdominal surgery quoted at 6% to 9% (Bradbury, 1997). (Surgery and CDI is discussed further in chapter 4).

These co-morbidities are thought to impair the immune response of the host making them more susceptible to CDI (Kyne *et al.*, 2002). In addition they propagate other risk factors by increasing the likelihood patients are on antibiotics, immune modulating agents and had recent or prolonged hospitalisation (McFarland *et al.*, 1990).

Duration of hospitalisation

The risk of acquiring CDI was found to be directly proportional to the length of stay in hospital (Johnson & Gerding, 1998). Colonisation rates in hospitalised patients were noted to be high between 14% and 20% (Kyne et al., 2002 and Johnson & Gerding, 1998). More recent colonisation rates are discussed further in chapter 5. Patients who were transferred between several wards were also found to be at increased risk of CDI (Starr *et al.*, 2003) as they were more likely to have prolonged hospitalisation and exposure to antibiotics.

Gastric acid suppression

Proton pump inhibitors PPIs are often suggested as risk factors for CDI (Dial *et al.*, 2006). By making the pH of gastric acid less acidic it was thought that *C. difficile* vegetative cells are able to survive and therefore increase the risk of infection. Their effects were demonstrated in animal models and the subsequent inflammatory response following infection in these animals was comparable to that of antibiotics (Kaur *et al.*, 2007). However several other studies have found either no link or a limited link between CDI and PPIs (Wilcox *et al.*, 2008, Muto *et al.*, 2005).

Other interventions and treatments

Chemotherapeutic agents, diuretics, opiates, laxatives and bowel preparatory agents have all been suggested to be associated with increased risk of CDI (Halim *et al.*, 1997; Raveh *et al.*, 2006; McFarland *et al.*, 1990, Faris *et al.*, 2010). Procedures such as nasogastric tube placement, enema administration and endoscopy have also been associated with an increased risk of CDI (Kyne *et al.*, 1999; McFarland *et al.*, 1990). This may reflect contamination of equipment with spores rather than decreased host defences mechanisms.

Strain type

The large variety of *C. difficile* strains can stimulate a varied inflammatory response in the patient and the extent of CDI is also dependant on the host's immunity. Certain hypervirulent strains notably ribotype 027 (Pepin et al., 2005) and more recently 078 (Jhung et al., 2008) have been reported on. Ribotype 027 was seen to cause increased disease severity and increased disease transmission. Other ribotypes 001 and 016 have also been associated with severe disease (Arvand et al., 2009).

1.7 The disease spectrum of *clostridium difficile* infection

The spectrum of *C. difficile* disease is varied with simple colitis being the most common form of symptomatic disease. The following disease spectrum categories are adapted from Bradbury, 1997; Dallal *et al.*, 2002 and Starr *et al.*, 2005. The more severe forms of CDI are discussed further in chapter 4.

1.7.1 Asymptomatic carriage

Asymptomatic carriage has commonly been reported in neonates and children and carrier rates in these populations varied from 4% to 60% (Holst *et al.*, 1981, McFarland *et al.*, 2000). Asymptomatic *C. difficile* carriage in healthy adults also occurs but not as commonly as in neonates and infants, with adult carriage rates varying from 3% to 7% (Kato et al., 2001; Kelly & Lamont, 1998). Asymptomatic carriage and its potential importance in the adult population are discussed further in chapter 5.

1.7.2 Simple colitis and severe colitis

Patients with simple *C. difficile* induced colitis develop mild to moderate watery diarrhoea which occurs greater than two to three times a day, and can be associated with colicky lower abdominal pain. The faeces produced is foul-smelling, due to the characteristic smell of *C. difficile*. Occasionally the diarrhoea may contain mucus and rarely blood.

Patients with severe colitis often have diarrhoea greater than five times per day and have more severe abdominal pain and abdominal distension can be present. They often have a leucocytosis and occasionally leucopaenia.

1.7.3 Pseudomembranous colitis (PMC)

Pseudomembranous colitis is one of the severest forms of CDI and is rare compared to the milder CDI forms. PMC is often a florid pancolitis but can occur segmentally and also eccentrically to involve the bowel wall. PMC gains its terminology from the classic yellow adherent plaques seen at sigmoidoscopy that can be scraped off the luminal surface of the bowel hence the term pseudomembrane. The pseudomembranous plaques can range in size from 2 to 10 mm or may be confluent.
As previously mentioned the pseudomembranes are made up of neutrophils, mucus, fibrin and sloughed epithelium. Patients with PMC often have severe abdominal pain and abdominal distension with a marked leucocytosis and hypoalbuminaemia. Diarrhoea is not always present.

Patients with typhlitis have a similar presentation and are severely immunosuppressed due to a pre-existing condition. The term typhlitis originally related to inflammation of the caecum only but has now become a generic term and includes the whole colon. These patients do not have pseudomembranous formation as they are neutropaenic.

1.7.4 Fulminant colitis

Fulminant colitis the most severe form of pseudomembranous colitis and is a very rare presentation of the disease. These patients are usually those that require surgical intervention for treatment. This presentation of CDI is discussed in more detail in chapter 4.

1.7.5 Small bowel enteritis

Spore germination is not affected by aerobic conditions (Wheldon et al., 2008), and therefore spores germinate within the small bowel. Patients who have ileostomies or ileo-anal pouches have been found to have CDI and in some cases pseudomembranous enteritis (Hayetian *et al.*, 2006). This is thought to occur due to histological changes in the ileal mucosa which results in a neo colonic environment (Mann *et al.*, 2003).

1.7.6 Community-acquired CDI

Although CDI is classically regarded as a nosocomial infection, community-related CDI cases are increasing and have been reported in up to 34% of cases (Kutty *et al.*, (2008). The European Centre for Disease Prevention and Control defined community acquired CDI as the onset of CDI symptoms outside a healthcare facility with the patient not having been discharged from a healthcare facility within 12 weeks of the onset of symptoms or the onset of symptoms within 48 hours of admission to a healthcare facility without a healthcare stay within 12 weeks preceding the symptom onset (Kuijper & van Dissel, 2008). Community acquired CDI can affect a younger age group (CDC, 2008) and often patients have no underlying risk factors or previous antibiotic exposure (Wilcox et al., 2008).

1.8 *Clostridium difficile* diagnosis

This section is described in more detail in chapters 4 and 5.

1.8.1 Clinical diagnosis

Clinical diagnosis is dependant upon the clinical symptoms of the patient and recognition of the disease and a patient's risk factors by health-care professionals. Following certain risk factors in particular exposure to antibiotics clinical suspicion for CDI should be high.

1.8.2 Laboratory diagnosis

This is most commonly done by detection of *C. difficile* toxins A and / or B in faecal specimens. Many laboratories use commercially produced enzyme immunoassay kits to detect any available toxins within the faecal samples. Those that test for both A and

B toxins should be ideally used due to toxin A-B+ *C. difficile* strains which can also cause symptomatic disease.

Further laboratory tests such as cytotoxic assay, toxigenic culture, glutamate dehydrogenase antigen detection, PCR assays and nucleic acid amplification tests are discussed further in chapter 5.

1.9 Treatment

1.9.1 Conventional therapy

In those patients with mild CDI stopping the inciting antibiotic may be all that is required. In 20% to 30% of CDI patients symptoms can resolve without further treatment (Starr, 2005; Johal et al., 2004).

For those patients requiring antibiotic therapy metronidazole, vancomycin, bacitracin, fusidic acid and teicoplanin have all been shown to be effective for CDI treatment (Nelson, 2007). Oral metronidazole and oral vancomycin are the most commonly prescribed antibiotics for CDI treatment. Metronidazole is usually commenced in the first instance for milder disease; this is partly related to cost and also due to the presence of vancomycin resistant enterococcus. Vancomycin has been found to be more effective in severe and recurrent disease (Sailhamer *et al.*, 2009), whereas increased failure rates and recurrence rates have been reported with metronidazole therapy (Pepin *et al.*, 2005). A combination of intravenous metronidazole and nasogastric/oral vancomycin is given to patients with the most severe forms of CDI (Lothian prescribing guidelines 2010).

1.9.2 New antimicrobials

Fidaxomicin is a new macrocyclic antibiotic for the treatment of CDI and it has been found to be superior to vancomycin for clinical cure and time to resolution of symptoms. The greatest use for this antibiotic is for those patients with recurrent disease as fidaxomicin has been shown to produce fewer recurrences (Poxton, 2010).

1.9.3 Probiotics and faecal microbiota transplants

Since disruption of the normal intestinal flora leads to CDI, restoration of the intestinal flora through replacement by other means has been considered as an alternative therapeutic option.

Generally only inconclusive evidence on the benefits of probiotics is available (Pillai & Nelson 2008) and on occasion probiotics have been the cause of fungaemia and bacteraemia in critically ill and immunocompromised patients (Tung et al., 2009).

Faecal microbiota transplants although less appealing have been performed with success to treat primarily recurrent CDI (Nood et al., 2009). This process uses healthy donor stool usually from a patient's relative which is administered via a nasojejenal tube or as an enema.

1.9.4 Non toxigenic *Clostridium difficile* strains

The aim of this therapy is to colonise the colon of patients who have been treated for initial episodes of CDI with non-toxigenic *C. difficile* strains to prevent re-infection with toxigenic strains. Trials are currently continuing to determine if this will help prevent recurrent CDI.

1.9.5 Vaccines

A vaccine containing toxoids A and B have been shown to resolve recurrent CDI symptoms in a study of three patients (Sougioultzis et al., 2005). Six months following vaccination the patients remained symptom free. Transcutaeneous injection with toxoid A in conjunction with cholera toxin in an animal model using mice induced both systemic and mucosal responses and may be a further immunisation technique (Ghose *et al.*, 2007).

1.9.6 Surgery

Surgery is usually only performed in cases of fulminant colitis, however mortality rates are high (Longo *et al.*, 2004). Prompt surgical referral for patients with severe CDI not responding to aggressive medical management is advised (Sailhamer *et al.*, 2009). Surgery for CDI is discussed in depth in chapter 4.

1.10 Prevention and control of *Clostridium difficile* infection

Prevention and control of CDI in healthcare facilities involves effective decontamination of the environment, good hand hygiene and barrier precautions and restriction of the use of antibiotics (Gerding *et al.*, 2008; Vonberg *et al.*, 2008) and all these strategies have been shown to reduce CDI rates. The prevention and control of CDI is discussed in greater depth in chapter 6.

1. 11 Aims

The main intentions of this thesis were to determine the local extent of CDI within hospitals and to establish the epidemiology of CDI within the specialty of colorectal surgery. This thesis consists of four studies, with their aims as follows:

1. Very limited information had been published on the burden of CDI to the diagnostic laboratory and the relative incidence of disease in different clinical specialties due to a paucity of suitable data. The study used locally collected data from 2000 to 2007 to assess the laboratory workload associated with *C. difficile* testing, and the potential clinical workload by calculating the maximal potential rates of CDI across all specialties and age groups. Severe outbreaks of *C. difficile* nosocomial diarrhoea caused by Type 027/BI/NAP1 had been seen worldwide since 2003 and dominated prevalence studies pertaining to *C. difficile*. This study provided data on an area that had not been affected by this strain during the period of the study.

2. This study used locally collected data to assess the potential impact of CDI within all surgical services as very few studies had compared the individual surgical specialties within the same urban population. In the absence of hypervirulent *C. difficile*, a further aim of this study was to assess if the more severe and extreme forms of *C. difficile* disease were occurring and to determine the proportion of patients affected and treated for the critical forms of CDI. The final aim of this study was to review the cohort of patients who had undergone surgical treatment for severe CDI.

3. The aim of this study was to prospectively determine the specific burden of *C. difficile* in the colorectal surgical patient population. This involved assessing the efficacy of hospital laboratory diagnostic testing methods for CDI and determining asymptomatic carrier rates and in-patient CDI rates within the colorectal surgical population. In addition the study aimed to provide epidemiological data on the strains of *C. difficile* and their antibiotic resistance patterns within this particular patient cohort.

4. This final study aimed to review the effect of *C. difficile* environmental surface contamination within the colorectal surgical wards. The study aimed to determine the extent of environmental *C. difficile* surface contamination and identify potential surface reservoirs for *C. difficile*. In addition the study was designed to assess changes in *C. difficile* surface contamination and the incidence of CDI within colorectal surgical in-patients following implementation of a new cleaning strategy. The final study objective was to provide epidemiological data of *C. difficile* strains found in the colorectal surgical wards surface environment and to compare them with those found in the colorectal surgical in-patient population.

2. Materials and Methods

2.1 Lothian database analysis for *Clostridium difficile*

2.1.1 Setting

Lothian University Hospitals Division (LUHD) provides services for around 620 000 people in the Edinburgh area of South-East Scotland (the second largest residential population in Scotland). Six of the major LUHD hospitals based within Edinburgh, comprising three tertiary and three secondary care hospitals and totalling approximately 2300 beds, were included in our analysis.

2.1.2 Laboratory diagnosis of *Clostridium difficile*

All faecal samples were processed by Lothian microbiology staff, in a single combined enteric microbiology laboratory for all the hospitals, following local guidelines prior to September 2006 and national guidelines after September 2006 (Health Protection Scotland, Protocol for the Scottish Surveillance Programme for *Clostridium difficile* Associated Disease, User Manual, Version 2.0, Revised October 2007). From 2003 to 2008 inclusive, all diarrhoeal (semi, unformed or liquid) faecal samples were tested if they were from hospital in-patients aged over 1 year old, if a diagnosis of antibiotic-associated diarrhoea or pseudomembranous colitis was present, on clinical request, or if the patient had been on recent antibiotic therapy. Appropriate faecal samples were tested for the presence of *C. difficile* toxins A and B via commercial enzyme immunoassay kits in accordance with the manufacturer's guidelines.

2.1.3 Inclusion Criteria

All in-patients from whom a faecal sample was submitted for *C. difficile* toxin testing were included in the data analysis. Patients were identified using the hospital laboratory computer filing system (Apex, iLab, Isoft, Banbury, UK) which stores details of each sample tested from 2000 to date. The data available included patient demographics, ward and speciality, where the patient was admitted at the time of sample collection, date of sample collection, test performed and results. The database

thus obtained, was filtered to exclude samples with indeterminate results, quality control samples, and those from hospitals which were not included in the study.

In line with national Scottish guidelines a new potential CDI episode was defined as "Only persons that have not been diagnosed with CDAD (*Clostridium difficile*-associated disease) within the previous 28 days are counted as new cases" (HPS 2007. Annual report on the surveillance of *Clostridium difficile*-associated disease in Scotland, October 2006-September 2007. The HPS *C. difficile* Working Group, December 2007). Therefore any repeat samples taken within a 28 day period following a positive toxin result for any individual patient were also excluded. Retrospective analysis of this prospectively collected data was then performed.

2.1.4 Potential *Clostridium difficile* infection rates

With recognition that every faecal sample that tests positive for *C. difficile* toxin does not always equate to symptomatic CDI, we have referred to the information gathered as potential CDI rates.

Potential CDI rates were calculated as potential episodes per 1000 in-patient occupied bed days (OBD) using statistics data obtained from the Information Analysis Department of the Health Intelligence Unit at NHS Lothian, courtesy of Ms Caroline Brown, on annual hospital occupied bed days from 2000 - 2007 per speciality. Data for renal medicine and transplant surgery had to be combined due to the combined ward set-up within Edinburgh from 2003. Therefore potential incidence rates for the renal medicine / transplant surgery specialty are only provided from 2003 – 2007.

The paediatric population i.e. under 18 years old, were excluded from the calculation of total potential CDI rates for the Edinburgh Lothian Hospitals, as paediatric age groups have been previously reported to have disproportionately elevated carriage rates (Tullus *et al.*, 1989 and Bryant *et al.*, 2009). This enables comparison of our results with other studies, which have only included adult populations, aged 18 and over.

2.1.5 Costs data

The most recent published data at the time of analysis was used to calculate potential associated costs (Vonberg *et al.*, 2008).

2.2 Colorectal surgical in-patient database analysis

2.2.1 Patient identification

The cohort of colorectal surgical in-patients identified from the total Lothian in-patient database analysis was assessed further.

In addition, patients who had required surgical treatment for severe CDI resulting in pseudomembranous colitis, over a ten year period from 1997 to 2006 inclusive, were identified using the Lothian Surgical Audit database and pathological data obtained from the Lothian hospital pathology filing system (APEX). 2007 data was not used as it was not available during the period of initial analysis.

2.2.2 *Clostridium difficile* diagnosis

Only those patients with a histo-pathological diagnosis of pseudomembranous colitis in their colectomy specimens between 1997 and 2006 inclusive were included in the database analysis surgical outcome sub-group.

For the surgically treated patient sub-group, clinical notes and the microbiology database were further scrutinised in order to obtain further clinical information.

2.3 *Clostridium difficile* carriers

2.3.1 Clinical setting and study participants

The Lothian colorectal service is primarily situated at the Western General Hospital in Edinburgh. The carrier cohort were recruited from those colorectal patients either attending the Western General Hospital colorectal service via the surgical out-patient clinic or those patients admitted for elective surgery including day-case and in-patient admission. Consent from the study participants was obtained by me.

2.3.2 Study group

Study participants were allocated a sequential identification number by Dr Allison Wroe as part of her Doctor of Philosophy thesis and this number was used to identify the faecal isolates.

Carriers were defined as those individuals who were asymptomatic and faecal sample culture positive for *C. difficile*.

2.3.3 Carrier faecal sample collection

Faecal samples were collected by me from asymptomatic participants during clinic appointments or on the day of admission for elective procedures prior to any surgical intervention. These samples were obtained in accordance with the terms granted and governed by the Local Research Ethics Committee.

2.3.4 Carrier faecal sample analysis

Analysis of these samples was performed by Dr Allison Wroe. Fresh faecal samples i.e. within 24 hours of collection were assessed for the presence of toxins A and B using a commercial ELISA kit (TechLab) in accordance with the manufacturer's protocol.

Culture of faecal samples and identification and characterisation of *C. difficile* was performed as per sections 2.4.3 to 2.4.5 and section 2.5.

2.4 Identification of *Clostridium difficile* in colorectal surgical in-patients

2.4.1 Clinical setting

In-patient colorectal services are situated at the Western General Hospital in Edinburgh. The in-patient colorectal surgical wards at the time of the study were wards 22, 23, 24 and 27. Ward 58 is the surgical High Dependency Unit. In-patient colorectal surgical boarders were situated in other speciality wards namely wards 56, 57 and 25.

2.4.1 Colorectal in-patient faecal sample collection

All faecal samples submitted to Lothian Hospitals microbiology enteric laboratory for *C. difficile* testing were processed following national guidelines. During the period of the study hospital diarrhoeal in-patient samples were tested by the enteric laboratory for *C. difficile* toxins A and B by enzyme immunoassay. All faecal samples submitted to the enteric laboratory from the colorectal surgical in-patient wards were reclaimed by me on a weekly basis. Any submitted faecal samples from colorectal in-patient surgical boarders on other wards were also reclaimed.

Faecal samples were reclaimed from the enteric laboratory from the period November 2007 to January 2009 inclusive.

The non identifiable patient faecal sample specimen number issued by the lab for each sample was maintained during the study to allow for continuity.

Samples were collected and analysed in accordance with the terms granted by the Local Research Ethics Committee. Due to the fact that samples were collected latterly from the enteric laboratory, any findings relating to a sample and hence a particular patient would therefore be retrospective and hence any findings of the study was not to impact on individual patient treatment.

2.4.2 Diarrhoeal Samples

All faecal samples collected were assessed for consistency using the Bristol Stool Chart. Dual observation for agreement between myself and Mr M. Baldock was used to Grade all the reclaimed faecal samples from 1 - 7 with the Bristol Stool Chart (Figure 2.1).

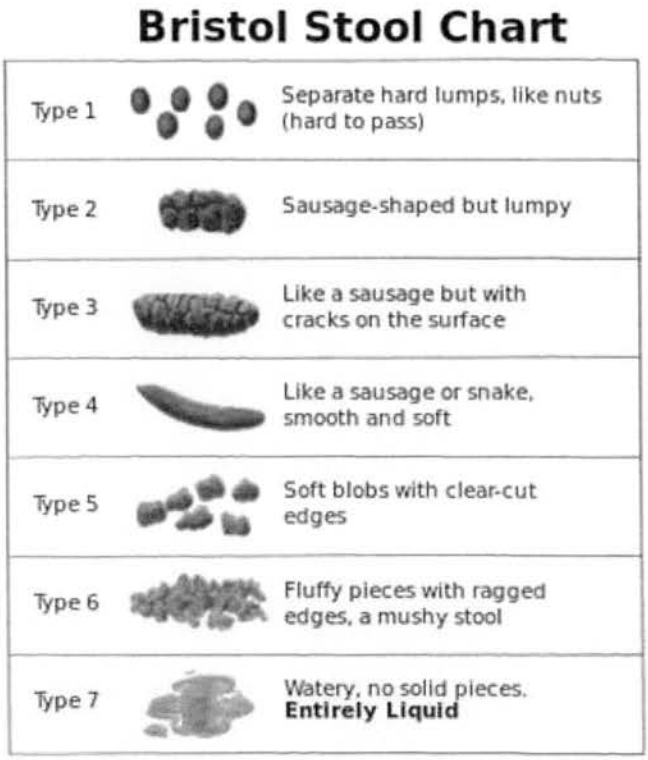


Figure 2.1 Bristol Stool Chart used for determining the consistency of faecal samples to identify diarrhoeal faecal samples.

2.4.3 Culture of faecal samples

All faecal samples reclaimed from the enteric laboratory were cultured on Brazier's CCEY (cefoxitin, cycloserine and egg yolk) agar (Oxoid) and incubated at 37°C under anaerobic conditions (10% hydrogen, 10% carbon dioxide and 80% nitrogen) in an anaerobic chamber (Don Whitley Scientific, Mark III station). These cultures were then reviewed at 24 hours, 48 hours and 5 days for the presence of *C. difficile* colonies.

All faecal samples that were *C. difficile* culture negative but identified as toxin positive by the enteric laboratory were re-cultured.

2.4.4 Identification of *Clostridium difficile*

C. difficile identification was made on characteristic colony morphology, smell and Gram stain. Colonies were assessed for characteristic chartreuse, golden-yellow,

fluorescence under a Wood's lamp (long wave UV light) and motility in a wet film by light microscopy.

2.4.5 Storage of *Clostridium difficile* isolates

A single colony only was isolated per culture positive sample and stored as spores in Robertson's cooked meat broth medium (Watt, 1973 and Brown *et al.*, 1996) at room temperature.

2.5 Characterisation of isolates

2.5.1 In-vitro detection of toxin production

C. difficile isolates obtained from the culture positive faecal samples and stored in Robertson's cooked meat broth were plated on blood agar (39g/L Columbia agar base, 5% defibrinated horse blood) and incubated anaerobically. The isolates were then sub-cultured into 3mls of pre-reduced proteose peptone, yeast extract medium (PPY; 20g/L proteose peptone, 5g/L yeast extract, 5g/L trypticase, 5g/L NaCl, 0.75g/L L-cysteine-HCl, 0.4g/L Na2CO3; pH7.1) and incubated anaerobically for 48 hours.

The growth medium for each isolate was then tested for *C. difficile toxins* A and B using Tox A/B II^{TM} (Techlab) kit. 1ml of the PPY culture supernatant was centrifuged at 16000 g for 1 minute, the supernatant was then diluted 5-fold in buffered diluent. The supernatant samples were then used to test for the presence of toxin in accordance with the manufacturer's protocol. Positive and negative controls were always tested with any samples. All isolates were tested in triplicate.

2.5.2 DNA extraction

Genomic DNA was extracted using two methods:

1. Using 1ml of *C. difficile* overnight cultures, these were centrifuged at 16000 g for 2 minutes. The NucleoSpin kit (Macherey-Nagel GmbH) was used to extract DNA by lysis of cells in a Proteinase K/SDS solution at 56°C for 3 hours. DNA was then bound to a silica filter using ethanol, following washing

with two buffers to remove contaminants; DNA was eluted using 100 µl of alkaline elution buffer.

2. Colonies grown on blood agar plates were suspended in a 5% Chelex-100 suspension (Bio-Rad Laboratories) and boiled for 10 minutes. The suspensions were centrifuged at 16000g for 2 minutes to separate out the DNA containing supernatant. The supernatant was aliquoted and stored at -20 °C.

2.5.3 Ribotyping

Using the method described by O'Neill et al., (1996) ribotyping was performed by amplification of the 16S-23S rRNA intergenic spacer region. The primers used were:
5'-CTGGGGTGAAGTCGTAACAAGG-3'
5'-GCGCCCTTTGTAGCTTGACC-3'
Amplification was performed in a 50µl reaction mixture containing 1.5 mM $MgCl_2$ 10Mm Tris-HCl, 50mM KCl, 2U of Taq polymerase (Promega), 200µM of each dNTP (Amersham Pharmacia BioTech), 50 pmol of each primer and 10µl of genomic DNA or sterile water for negative controls.
The thermal PCR profile used involved initial denaturation at 94°C for 4 minutes followed by 35 amplification cycles of denaturation at 96°C for 1 minute; annealing at 56°C for 1 minute and extension at 72°C for 1 minute, with a final elongation step at 72°C for 5 minutes. The PCR products were concentrated by heating at 45°C for 100 minutes. A 3% Metaphor gel (ABgene) containing 30µl of Safe View Nucleic Acid Stain (NBS Biologicals) was used for the product electrophoresis at 80V for 3 hours, in conjunction with a 100 base pair ladder (Promega). The final gels were photographed under UV light and the band patterns analysed by Bionumerics Gel Compar software which compared them with those held on the local laboratory database to determine the ribotypes.

Limited ribotyping was performed by myself on 20 isolates. The main body of ribotyping data was produced by Dr M Desai under the direction of Prof H Shah at the Health Protection Agency, Colindale, London; by use of a multi-analyser system used for multilocus variable number tandem-repeat analysis (MLVA) typing of a number of bacterial and viral organisms. *All C. difficile* isolates obtained during the study were transported to Colindale on charcoal swabs.

2.5.4 Toxinotyping

Using the method described by Rupnik *et al.*, (1997; 1998) toxinotyping was performed. The fragments of *tcd*A (A3) and *tcd*B (B1) genes were amplified by PCR. The primers used to amplify the B1 fragment are:

5'-AGAAAATTTTATGAGTTTAGTTAATAGAAA-3'

5'-CAGATAATGTAGGAAGTAAGTCTATAG-3'

The primers used to amplify the A3 fragment are:

5'-TATTGATAGCACCTGATTTATATACAAG-3'

5'-TTATCAAACATATATTTTAGCCATATATC-3'

The amplified PCR products were then digested by restriction enzymes.

Amplification was performed in a 50µl reaction mixture containing 1.5mM $MgCl_2$, 10mM Tris-HCL, 50mM KCl, 0.5U of Taq polymerase (Promega), 200 µl of each dNTP (Amersham Pharmacia Biotech), 50pmol of each primer and 5µl of genomic DNA or sterile water for negative controls. Amplification of the A3 fragment reaction mixture also contained 5mg bovine serum albumin (BSA, Sigma-Aldrich), 0.05% W1 and TMA (tetramethylammonium chloride, Sigma-Aldrich) with a final concentration of 1mM.

The thermal PCR profile used involved initial denaturation at 93°C for 3 minutes, followed by 35 amplification cycles involving annealing and extension at 47°C for 8 minutes; denaturation at 93°C for 4 seconds and a final extension at 47°C for 10 minutes. PCR products were concentrated by heating at 75°C for 45 minutes. Restriction was performed for A3 using 10µl of product and 2µl of the enzyme EcoRI and for B1 20µl of product was digested using the enzymes HincII, 1µl, and AccI, 1µl. (Enzymes were obtained from New Engand BioLabs). Incubation at 37°C for 2 hours allowed completion of the restriction fragment length polymorphism step. The final fragments were visualised on a 1% agarose gel which had been placed in Tris-borate-EDTA buffer (TBE) containing 10µl of SafeVeiw nucleic acid stain (NBS Biologicals). The toxinotypes were determined by reference to the Rupnik *et al.*, papers.

2.6 Antibiotic Susceptibility testing

2.6.1 Antibiotics

Antimicrobial susceptibility testing of the *C. difficile* isolates was performed using four antibiotics; ceftriaxone, ciprofloxacin, metronidazole and vancomycin. These antibiotics were chosen as metronidazole and vancomycin were used to treat CDI, ceftriaxone was the commonest used antibiotic along with metronidazole in colorectal surgery at the time of this study and fluoroquinolone use was being increasingly used in other specialties. The concentrations tested and the guidelines for minimum inhibitory concentration interpretation were adapted from the Clinical Laboratories Standards Institute Guidelines criteria for anaerobes (Mutlu *et al.*, 2007), and are as follows:

Antibiotic	Range of concentrations tested (μg/ml)	Susceptible (μg/ml)	Intermediate (μg/ml)	Resistant (μg/ml)
Ceftriaxone	16.0 – 256.0	≤16	32	≥64
Ciprofloxacin	4.0 – 128.0			≥16
Metronidazole	0.5 – 8.0	≤8	16	≥32
Vancomycin	0.5 – 8.0	≤2	4 - 16	≥32

2.6.2 Minimum inhibitory concentration (MIC)

The Wadsworth agar-dilution method was used to assess the minimum inhibitory concentrations for each antibiotic. For two isolates where the MIC could not be clearly determined for Metronidazole E-test strips were used.

The *C. difficile* isolates were sub-cultured from the Robertson's cooked meat broth stores and grown on blood agar. Following anaerobic incubation at 37°C for 24 to 48 hours, a minimum of five colonies were inoculated into 3mls of pre-reduced thioglycollate medium (Oxoid) enriched with 5μg/ml haemin, 1μg/ml vitamin K_1 and 1mg $NaHCO_3$ ml^{-1}, which were incubated anaerobically overnight at 37°C. The turbidity of the cultures were adjusted to an optical density of 0.5 McFarland standard by the further addition of pre-reduced enriched thioglycollate broth. Aliquots of the cultures were spotted under aseptic technique using a multipoint inoculator onto pre-

reduced Brucella agar (Oxoid) supplemented with haemin 5μg/ml, 5% lysed horse blood, vitamin K_1 and 2ml of a given concentration of an antibiotic. The antibiotic stocks (10000μg/ml) were diluted ten-fold to the maximal initial concentration to be tested, then two-fold stock dilutions were prepared using distilled water until the ten-fold minimal concentration per antibiotic to be tested was attained. The lowest concentration plate for each set of antibiotics was inoculated first. Two quality control plates prepared with 2mls of distilled water to replace the antibiotic agent were also inoculated prior to and a further two control plates after each antibiotic set. The control plates were then separated for aerobic and anaerobic incubation to assess for contamination. The *C. difficile* reference strain 630 was used as a control strain, this strain belongs to ribotype 012 and represents an historic isolate in Scotland, with its MICs available from a previous study with the exception of ciprofloxacin (Drummond *et al.*, 2003). Growth on the plates was assessed at 24 and 48 hours of incubation and the MIC of each antibiotic for each isolate were determined by the lowest concentration of antibiotic to inhibit visible bacterial growth. All isolate MIC testing was repeated in triplicate.

The E-test method was performed on two isolates where despite triplicate assessment the MIC for metronidazole could not be accurately determined. Isolate cultures were swabbed onto the pre-reduced enriched Brucella agar plates to obtain a lawn of growth. Metronidazole test strips were kindly donated by the enteric laboratory of NHS Lothian, these were impregnated with an exponential gradient of antibiotic and were smoothly placed on each isolates' plate. The plates were incubated anaerobically at 37°C and assessed at 24 and 48hours. The MIC was determined by the antibiotic concentration on the strip where the isolate growth zone of inhibition began.

2.7 Environmental Contamination of *Clostridium difficile*

2.7.1 Setting

The acute surgical wards with colorectal surgical patients at the Western General Hospital Edinburgh with the exception of the surgical High Dependency Unit were sampled for the presence of *C. difficile*. The wards sampled were 22, 23, 24 and 27, comprising 80 beds. Ward 22 at the start of the study became primarily a day-case

with overnight stay ward for both Colorectal and Urology surgical patients. Please note these were the same wards from which patient faecal samples were reclaimed from the enteric laboratory.

2.7.2 Environmental Sampling

During the period January 2008 to March 2009 inclusive, 180 Brazier's CCEY contact plates were used to sample the aforementioned colorectal surgical wards environment approximately every six weeks, with the first sampling session in January 2008 commenced at the end of the month. The same areas were sampled on each occasion using the same method, in the same manner to provide consistency. The surfaces were sampled by touching the domed inverse meniscus agar of the contact plate to the required surface. The plates were labelled in conjunction with the sampling process. Following completion of sampling within the hospital environment, the plates were transported by car to our laboratory. The contact plates were incubated within the anaerobic chamber at 37°C and assessed at 48 hours and 5 days for *C. difficile* growth. Any *C. difficile* isolates were identified and assessed as per previous sections 2.4.4 and 2.5.

A new cleaning regime was introduced by NHS Lothian on 3rd June 2008, six months into the study. The new cleaning protocol was integrated into an existing infection control pathway. Once a patient with diarrhoea was identified in a shared main bay, the patient was isolated in a side room and a faecal sample was to be obtained for microbiology testing. The new cleaning protocol involved requesting a terminal clean of vacated bed spaces and the surrounding environment with Actichlor plus (sodium dichloroisocyanurate (NaDCC)) at a concentration of 1000ppm and a bed space curtain change. Once the patient was isolated, the room door was to be kept closed and standard infection control precautions implemented. Soap and water only was to be used for hand hygiene and gloves and apron for contact with the patient and their room environment. A daily clean with Actichlor plus, again at 1000ppm, of the isolated symptomatic patient's room environment and equipment was to occur. Isolated patient's also had disposable equipment for the duration of their isolation such as blood pressure monitor (sphygmomanometer) cuffs.

Actichlor plus replaced the standard detergents of a quaternary ammonium based compound and or bleach (sodium hypochlorite (NaOCl) the dilution of which was variable between 1000 to 3000ppm) for deep cleaning.

2.8 Ethics approval

Permission for samples obtained to determine the asymptomatic carrier rate was provided by the Lothian Local Research Ethics Committee. An addendum to the provided ethical approval was also submitted and granted in regards to reclamation and assessment of faecal samples from the enteric laboratory with no results obtained to interfere with individual patient management as the faecal samples would be retrospectively collected weekly. Review of any patient clinical data was performed by me as stipulated in the given ethical approval. Local Caldicott approval was given for review and analysis of the microbiology databases. Review and analysis of the surgical and pathological databases, in addition to review of patient data was covered by ethical approval, local ethics and local audit procedures and guidelines.

Environmental sampling was performed following permission of the Colorectal Surgical Team including consultants and charge nurses in addition to the Infection Control Team at the Western General Hospital at the time of the study. Environmental sampling was only performed when it would not interfere with any patient care or privacy and therefore verbal consent was also taken for each patient prior to sampling of their bed area.

2.9 Statistical Analysis

All statistical analysis was performed using the GraphPad Software Prism 4.0. Data were analysed using the Student's unpaired t-test and the Kruskal-Wallis one-way analysis of variance (ANOVA) testing. P values of ≤ 0.05 were considered statistically significant.

3. Changes in local trends, laboratory and clinical workload for *Clostridium difficile* infection from 2000 – 2007.

3.1 Introduction

The incidence of *Clostridium difficile* infection has increased particularly in the industrialised world over the past three decades. CDI is the principal cause of nosocomial diarrhoea in adults in these countries and is an important cause of antibiotic-associated diarrhoea.

Very limited information had been published on the burden of CDI to the diagnostic laboratory, and the relative incidence of disease in different clinical specialties was not well known at the time of this study. This was largely due to the paucity of suitable data. In Scotland voluntary data submission of CDI was present from 1984, with reporting of CDI becoming mandatory for those aged 65 years and over in September 2006.

The study aimed to use locally collected data from 2000 to 2007 to assess the laboratory workload associated with *C. difficile* testing and to analyse the maximal potential rates of CDI across specialties and age groups, thus providing information on potential clinical workload. Severe outbreaks of *C. difficile* nosocomial diarrhoea caused by Type 027/BI/NAP1 have been seen worldwide since 2003. However, this chapter provides data on an area that has not been affected by this strain during the time period of the analysis. To our knowledge this was one of the first studies to provide information on laboratory workload associated with *C. difficile* testing.

The paediatric population i.e. under 18 years old, were excluded from the calculation of total potential CDI rates for the region to enable comparison of our results with other studies, which have only included populations aged 18 and over. Therefore most potential CDI rates quoted are for the adult in-patient population unless otherwise stated.

3.2 Results

Over the analysis period a total of 50589 faecal samples were tested for *C. difficile* toxin, of these 7294 were positive (14.4%). After excluding repeat positive samples from the same patient in a 28-day period, 5909 new potential episodes of CDI were identified in the period 2000-2007, increasing from 112 potential cases in 2000 to 1231 in 2006 (Figure 3.1a). The overall rate of potential CDI for in-patients admitted over this period was 1.52 cases per 1000 in-patient occupied bed days (OBD), increasing from 0.36 potential cases/1000 OBD in 2000 to a peak of 1.98 potential cases/1000 OBD in 2006 (Figure 3.1b). Although both graphs follow different patterns; both the potential number and rate of CDI episodes fell in 2007 to 928 and 1.48 cases/1000 OBD, respectively.

Laboratory Workload

The number of faecal samples tested for *C. difficile* increased from 526 in 2000 to 14207 in 2006 falling to 10359 in 2007. Although the proportion of these samples identified as positive has decreased from 28% in 2000 to 11% in 2007, with the proportion of positive samples from 2003 to 2007 varying annually from 11% to 16% (Figure 3.2a). There was an almost equal division of samples sent from Medicine of the Elderly, all Medical Specialties and all Surgical Specialties. Medicine of the Elderly was the specialty which sent the greatest number of samples for analysis overall and was almost double the proportion of samples sent by the next three specialties individually; these were General Surgery, Gastrointestinal Medicine and Infectious Diseases. Medicine of the Elderly had the greatest proportion of positive samples (24%) followed by Urology (17.7%), Renal Medicine and Transplant Surgery (16.8%), Respiratory Medicine (15.7%) and General Surgery (15.5%) (Figure 3.2b).

Stratifying the data according to age, the greatest proportion of samples was sent from the 61-80 year old age group and the highest positivity rate was seen in those aged over 80 years. One third of all samples were sent from those aged 60 years or younger and 18.5% of all positive samples came from this age group (Table 3.1).

Clinical workload

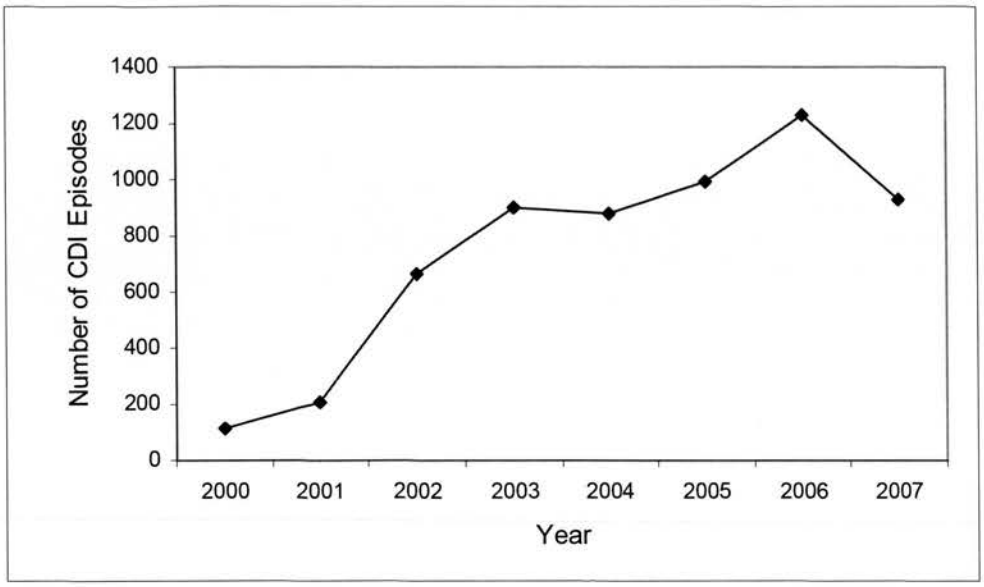
Renal Medicine/Transplant Surgery with 7.36 cases/1000 OBD and Intensive Care and High Dependency (ITU+HDU) with 5.73 cases/1000 OBD had the highest potential incidences of CDI followed by Infectious Diseases and Gastrointestinal Medicine whose rates were 4.75 and 4.26 cases/1000 OBD, respectively. Medicine of the Elderly in comparison had an incidence of 1.95 cases/1000 OBD (Figure 3.3a).

On review of the trends in potential CDI incidence of the seven specialties with the highest rates over the 8-year time frame (Figure 3.3b), all latterly had a reduction in potential CDI rates despite following varying trends. Medicine of the Elderly, Infectious Diseases and Gastrointestinal Medicine appeared to have the greatest rate reduction. For each specialty the potential CDI rates peaked at different time points. Renal Medicine and Transplant Surgery had a maximal rate in 2006, ITU+HDU in 2004, Infectious Diseases and Gastrointestinal Medicine in 2005, Medicine of the Elderly in 2006, General Surgery in 2003 and General Medicine in 2002.

The incidence of potential CDI rates also increased with age, over the total study period, from 0.33 cases/1000 OBD in the 0-20 years old age group to 1.81 cases/1000 OBD in the 61-80 years old age group. Similar to the specialty trends, all age groups latterly saw a reduction in rates. The potential peak incidence occurred in 2002, 2003 and 2006 for those aged 41 years and over, with the peak incidence in the younger two age groups, occurring later; in 2005 in the 21-40 years old age group and 2006 in the 0-20 years old age group (Figure 3.4).

Of all toxin-positive patients 10.3% were transferred through a minimum of two specialties and 17% of all these inter-specialty transferred patients were transferred through a minimum of three to a maximum of six specialties. These transfers also included movement through acute service areas which comprise Accident and Emergency, Combined Assessment Units and Acute Receiving Units.

a)



b)

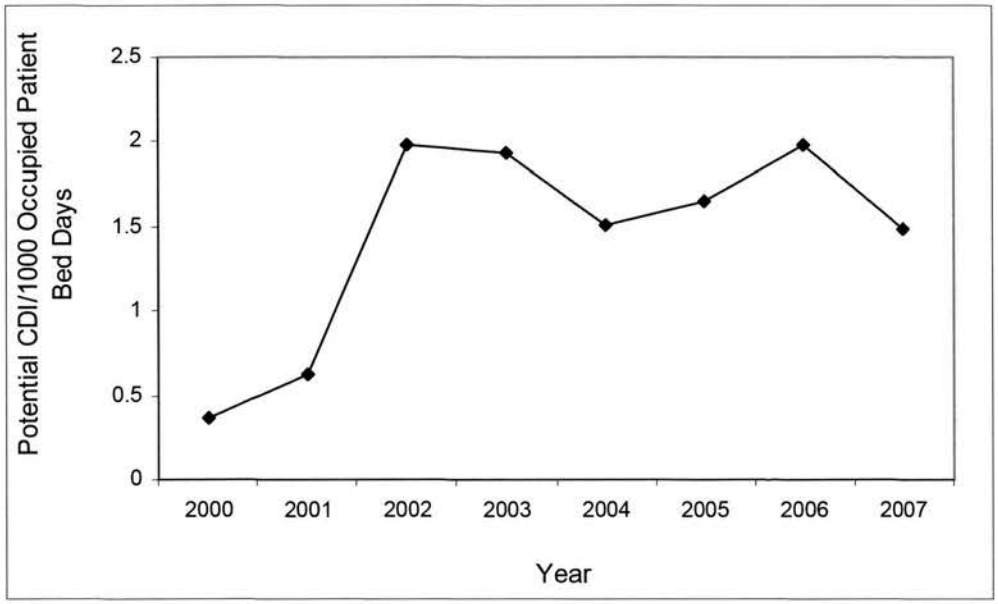
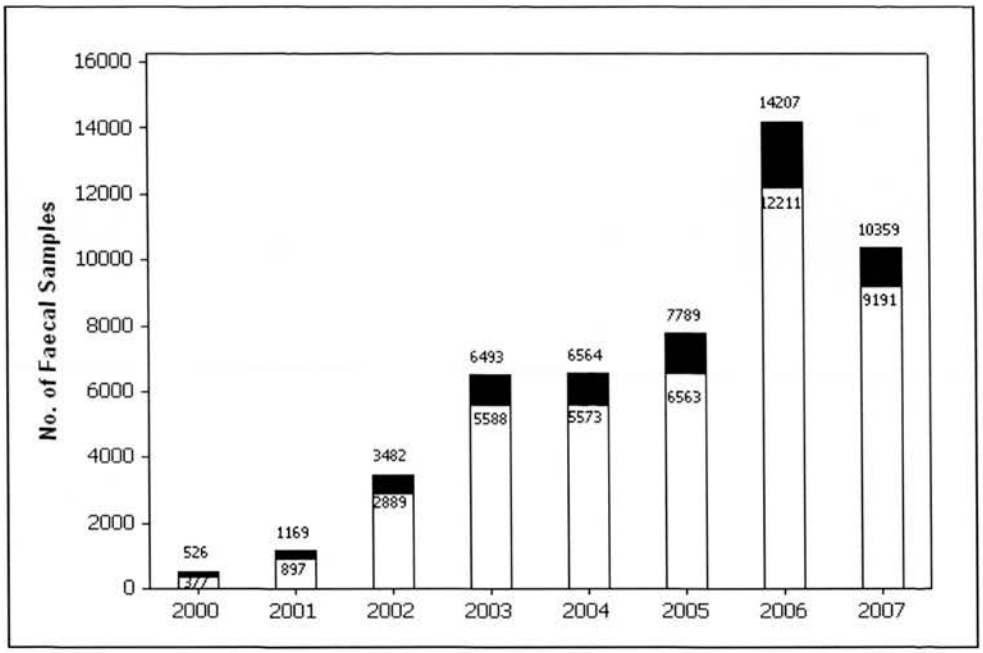


Figure 3.1 Difference between the changing incidence and number if CDI episodes from 2000-2007.

a) Total number of episodes of CDI per year (as defined by a stool sample positive for *C. difficile* toxin). In the case of multiple positive samples from the same patient within a 28-day period only one was included in the total.
b) Changes in the incidence of CDI episodes per 1000 OBD.

a)



b)

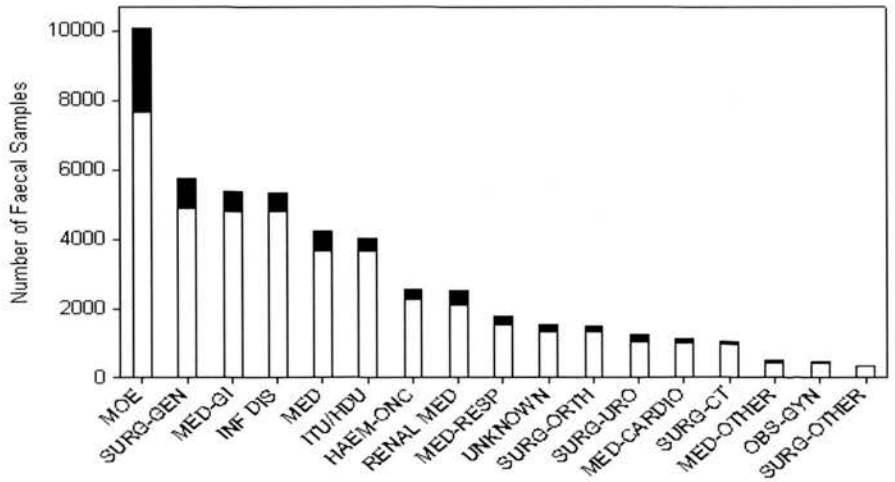


Figure 3.2 Trends in number of laboratory requests for *Clostridium difficile* toxin detection 2000-2007. a) Total number of lab requests *C. difficile* detection per year and b) Total number of lab requests for *C. difficile* distributed by specialty.

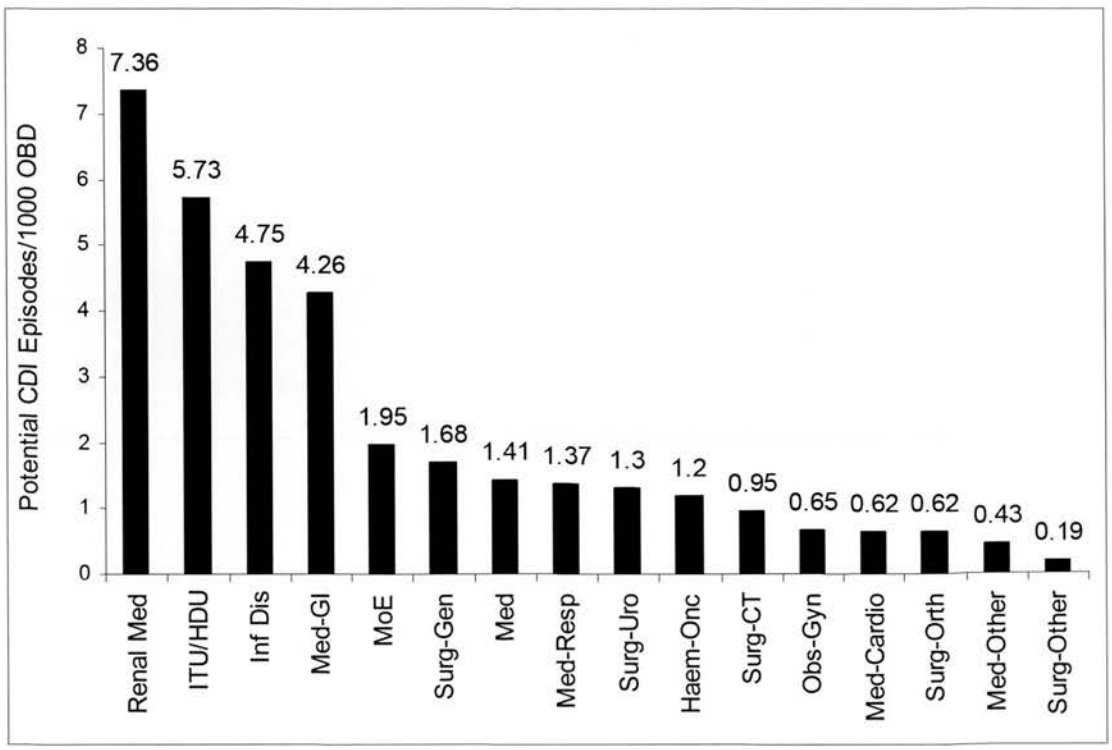
Shaded areas: positive results for *C. difficile* toxin; non-shaded areas negative results for *C. difficile* toxin. HAEM-ONC: Haematology and Oncology; INF-DIS: Infectious

Diseases, ITU/HDU: Intensive Care and High Dependency Units; MED: General Medicine; MED-CARDIO: Cardiology; MOE: Medicine of the Elderly; MED-GI: Gastrointestinal Medicine; MED-OTHER: includes all other medical specialties including dermatology and rheumatology; MED-RESP: Respiratory Medicine; RENAL MED: Renal Medicine and Transplant Surgery; SURG-CT: Cardiothoracic Surgery; SURG-GEN: General Surgery; SURG-ORTH: Trauma and Orthopaedic Surgery; SURG-OTHER: includes all other surgical specialties including vascular, ENT, plastics and maxillo-facial ; SURG-URO: Urology; UNKNOWN: Samples that could not be allocated to a particular specialty.

Age range	Number of samples (%)	
	Tested	Positive
0-20	2,060 (4.1)	104 (1.4)
21-40	5,092 (10.1)	344 (4.7)
41-60	9,570 (18.9)	905 (12.4)
61-80	20,272 (40.1)	3,196 (43.8)
>80	13,595 (26.9)	2,745 (37.9)
Total	50589 (100)	7294 (14.2)

Table 3.1 Number of samples tested (total and proportion positive) by the laboratory distributed according to age group

a)



b)

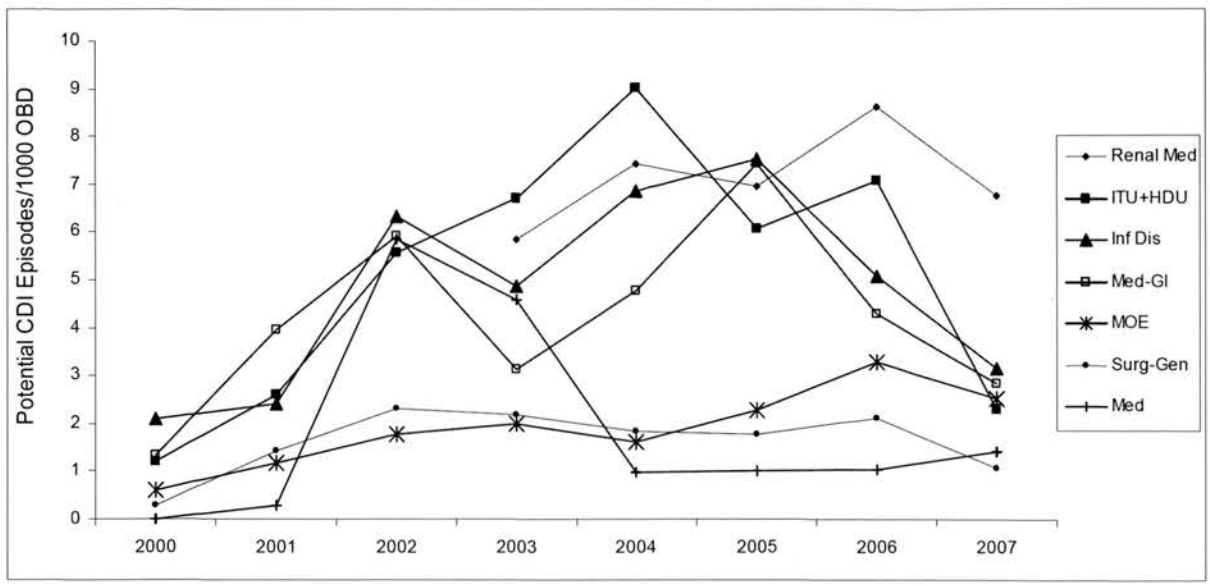


Figure 3.3 Distribution of CDI incidence among the various specialties from 2000-2007. a) The overall incidence of CDI episodes per 1000 occupied patient bed days per specialty and b). Annual incidence of CDI in the seven specialties with the highest overall incidence of CDI (episodes per 1000 occupied patient bed days). (Data for Renal Medicine and Transplant Surgery available from 2003 onwards only), Key as for figure 3.2.

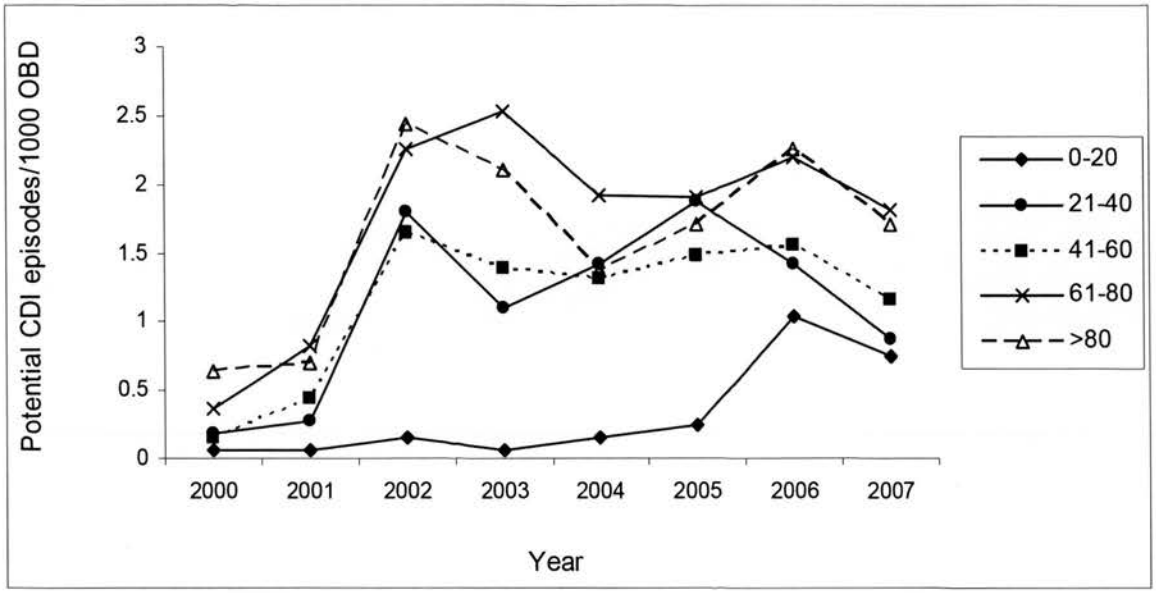


Figure 3.4 Distribution of annual age-related CDI incidence (episodes per 1000 OBD) from 2000-2007.

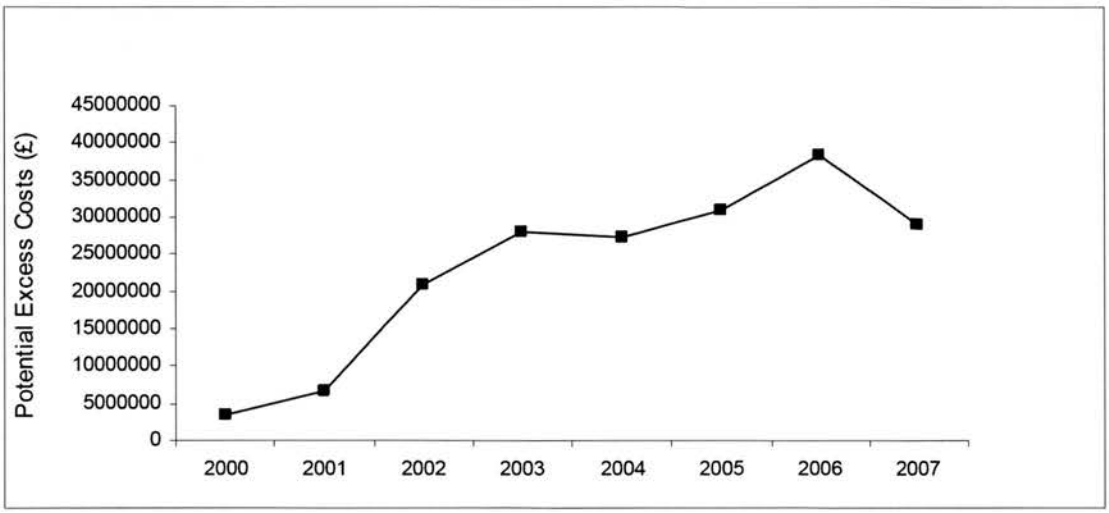


Figure 3.5 Excess potential costs of CDI in-patient cases per year.

3.3 Discussion

The *C. difficile*-related burden handled by the diagnostic microbiology enteric laboratory and the potential clinical workload over an 8-year period within this region of Southeast Scotland (2000-2007 inclusive) were assessed. The results represent the first study from Scotland, and possibly the first ever, demonstrating CDI laboratory workload, with recent CDI trends and maximal potential specialty-dependent clinical CDI rates also reported. Although the study reviews presumptive rates of specialty based CDI based on toxin positive faecal samples rather than symptomatic defined disease, the study still provides valuable information on the burden of disease prior to mandatory data reporting, introduced in September 2006, and for age groups below 65 years.

Despite the total number of potential CDI cases increasing from 2000-2006 followed by a decline in 2007, a fall in the potential rate of CDI occurred between 2003 – 2004 and 2006 – 2007, following an initial marked increase from 2000-2002. This difference could reflect an increase in the number of patients being treated as an in-patient. The initial reduction in CDI rates may be the result of increased awareness and improved infection control practices with the subsequent rise occurring if infection control measures relaxed with an associated increase in antibiotic usage (e.g. fluoroquinolones). The decline in CDI rate seen latterly is likely to represent a true decline as this reduction occurred throughout all specialties and may represent enhanced infection control measures including improvements in CDI-related education and antibiotic stewardship. This reduction is converse to some other European and international reported CDI incidence figures. In Canada, an increase in incidence from 2.5 cases/1000 admissions to 4.6 cases/1000 admissions occurred from 2002 to 2007 (Gravel et al 2009). In Spain where ribotype 027 is not prevalent (as in our study), the prevalence rates have increased from 0.39 to 1.22 cases per 1000 hospitalised patients from 1999 to 2007 (Asensio et al 2008). The incidence of 1.27 CDI cases/1000 OBD in the whole of Scotland from October 2006 to September 2007 for those aged 65 years and over (HPS December 2007) was lower than the 1.99 CDI cases/1000 OBD incidence found in this study for the same period and patient cohort. This may be a result of our study representing potential CDI in a concentrated urban population.

There has been a 27-fold increase in the number of faecal samples analysed by the diagnostic microbiology laboratory from 2000-2006, with a 54% increase in the number analysed from 2003 to 2006. Although the number decreased from 2006 to 2007, there was a 37% increase from 2003 to 2007. The overall increase will have impacted upon staff requirements including appropriate analysis of samples, timely data reporting, storage of samples and further testing of repeat samples sent from previously positive patients. In addition this increase will also have had implications for ward-based staff with regards to rapid and appropriate isolation of patients, cleaning procedures and continued patient management. Local guidelines prior to 2008 required all in-patient diarrhoeal samples to be tested for *C. difficile* toxin. Since then in an attempt to reduce laboratory workload, diarrhoeal samples sent from those aged 16 years and under are only tested for *C. difficile* if specifically requested.

One third of samples tested were submitted from those aged 60 years and under, and this population also accounted for approximately one-fifth of total positives (Table 3.1). This was similar to the results from the 2009 European Study (Bauer *et al.*, 2011) where 37% of samples were submitted from those aged 65 years or under. In the Netherlands in 2005, 42% of samples were submitted from the same age group (Paltansing *et al.*, 2007). With the exception of those aged under 20 years, all the remaining age groups followed similar trends over the study period with an exponential increase from 2000-2002 followed by a smaller variable incidence change of 10 potential CDI episodes/10000 OBD between 2002-2006. In this study, CDI toxin positivity was identified in all age groups including those aged 0-20 years, and the potential incidence in those aged 21-40 and 41-60 years was not largely dissimilar from those aged over 60 years. It should be noted that the potential false positive rates will be higher among the younger age groups because the potential prevalence in these age groups i.e. aged 60 years and under is <10%. With mandatory data reporting in Scotland initially introduced for those aged 65 years and above only, a large proportion and important subset of patients were being excluded from national figures. Subsequent to May 2009, mandatory data reporting in Scotland now includes those aged 16 years and above.

Traditionally the elderly population has been deemed high risk for CDI; however, it is no longer the only at risk group (McFarland *et al.*, 2007). An analysis of our data by

specialties revealed that Obstetrics and Gynaecology patients, a group with minimal CDI association, have a low but noteworthy incidence of disease. The CDI rate in Obstetrics and Gynaecology was found to be greater than Orthopaedics an erstwhile higher risk population for CDI development due to an older patient base. Because there is only a few reports of CDI in peripartum women (MMWR 2005; Venogopal., *et al.*, 2011), in general a young patient cohort, they, along with paediatric patients comprise a sub-group where there should be vigilance and a high index of clinical suspicion in symptomatic patients.

Medicine of the Elderly accounted for the largest number of samples tested by the diagnostic laboratory for a single specialty which was almost double the proportion of samples sent by the next three specialties individually (General Surgery, Gastrointestinal Medicine and Infectious Diseases). Therefore it is of interest to note that Medicine for the Elderly did not have the highest potential rate of CDI. Advanced age has been found to be an independent predictor of mortality in CDI patients (Ang et al 2008). However, in our study, Medicine of the Elderly, had a much lower incidence than several other specialties, although it provided the maximal specialty laboratory workload and had the greatest proportion of positive samples. Renal Medicine/Transplant Surgery, ITU, Infectious Diseases and Gastrointestinal Medicine all had higher incidences of CDI. This is similar to a study from Amsterdam where CDI occurred more frequently in ICU patients and those admitted to surgery (Kuijper et al. 2001). Conversely however, in Spain, admission to a general medical ward has been associated with an increased CDI prevalence as opposed to an ITU admission (Asensio et al 2008). All the aforementioned specialties had a peak incidence at different time points followed by a sustained decline. This may represent heightened awareness within that particular specialty during peak periods resulting in improved infection control measures which were maintained.

Renal Medicine/Transplant Surgery had the highest incidence of CDI over the study period. This may reflect the overall antibiotic usage, immunosuppression and required repeated admissions in this population. Chronic haemodialysis patients have a greater risk of developing nosocomial infections including CDI (D'Agata et al 2000) and renal insufficiency is associated with increased risk of CDI following live donor liver transplantation (Hashimoto et al. 2007). Liver disease is also independently associated

with severe CDI outcome (Gravel et al 2009) adding to the risk of CDI development in this cohort of patients which includes both renal and hepatic transplant patients. OBD figures were only available from 2003 onwards in this group and it is probable that the overall rate may have been slightly lower if calculated from 2000.

Recognition of patients with severe CDI, as a result of increased awareness following the Canadian CDI outbreaks in 2002 (Pépin et al, 2004) and the Stoke Mandeville (Bucks, UK) outbreaks from 2003 to 2005 (Healthcare Commission. Investigation into outbreaks of *Clostridium difficile* at Stoke Mandeville Hospital. London: Buckinghamshire Hospitals NHS Trust, 2006) could have resulted in an increased number of patients being referred to ITU and hence the observed peak in 2004. The marked decline in potential CDI incidence observed afterwards within ITU and HDU areas may be a result of increased awareness, stringent infection control and the overall decrease seen across all specialties. The potential incidence of CDI, in intensive care and high dependency patients in this study was 5.73 cases /1000 OBD. Lawrence *et al.* (2007) found the incidence of CDI acquisition in ITU to be 3.2 cases/ 1000 patient days in the absence of an outbreak. This difference may be a result of differing patient populations and our figure represents a maximal potential incidence, which includes those patients transferred to ITU with CDI. A specific subgroup of young critically ill trauma patients admitted to ITU had been found to develop CDI even though administration of antibiotics was for surgical prophylaxis only (Lumpkins *et al.,* 2008). This may have also contributed to the overall rate of incidence in the ITU & HDU group.

The high incidence associated with patients in Infectious Diseases could be the result of direct referral of symptomatic patients to the service from the community or transfer of patients to Infectious Diseases from other specialties.

Gastrointestinal Medicine may handle a substantial burden of CDI as a result of increased prevalence of CDI among patients with inflammatory bowel disease. CDI has been reported to be eight times more prevalent in patients with ulcerative colitis and five times more prevalent in patients with Crohn's disease affecting the colon compared to the general population (Ricciardi *et al.*, 2009 and Nguyen *et al.*, 2008).

General Surgery had a greater overall incidence than General Medicine and was the only specialty where a plateau in incidence occurred from 2002-2006 despite a rise in the total CDI incidence in 2006. This is in contrast to other studies which have noted a rise in CDI among patients admitted in surgery, particularly for patients requiring surgical intervention for CDI (Longo *et al.*, 2004, Koss *et al.*, 2006). This is examined further in Chapter 4.

The potential incidence of CDI in the 0 – 20 year age group exponentially rose from 0.059/1000 OBD to a peak rate of 1.04/1000 OBD in 2006. Kim *et al*,. (2008) similarly demonstrated a rise in incidence in a similar population (0 – 18 years) of treated CDI patients over a similar time period with an incidence rise from 4.4 cases / 10000 patient days (0.44 cases/1000 patient days) in 2001 to 6.5 cases / 10000 patient days (0.65 cases/1000 patient days) in 2006. The difference in the 2006 incidences may be due to an over-estimation in our cohort as our study intimates potential rates and the paediatric age groups have been previously reported to have disproportionately elevated carriage rates (Tullus *et al.*, 1989 and Bryant *et al.*, 2009). Also the 18 -20 year old group was included in our cohort. Due to the small but notable rise in figures in this low-risk group, the paediatric data i.e. those aged less than 18 years, was assessed further.

Of the total faecal samples 4.1% of all samples were submitted from the 0 to 20 year old age group (table 1) to the enteric laboratory for testing (table 3.1) of these 3.1% were from those aged less than 18 years old, with 4.6% of samples sent from the paediatric population testing toxin positive. Taking into account multiple samples testing for individual patients, 51 paediatric patients were identified as toxin-positive, with the majority of toxin positive patients identified between 2006 -2007; with 23 toxin positive patients in 2006 and 20 in 2007. Of these 51 patients there was no gender bias, male:female = 25:26, however the median age was 4 years (range 2months to 17 years). This young median age in the paediatric CDI population is not uncommon; Rexach *et al.,* (2006) noted average ages of 3.5 and 5.3 years, and Kim *et al.*, (2008) also noted a median age of 4 years in their study of 4895 paediatric patients with CDI. A range of 1 to 31 faecal samples were tested per patient, for the toxin-positive paediatric cohort, over a range of 1 – 133 days. Only 54% of these patients were found to be toxin positive on their first sample. A median period of 17

days (range 2 to 102 days) was conceded, for the remaining 46% prior to the detection of *C. difficile* toxins, from the time of their first negative sample being assessed to the positive sample, with a median of 3 samples sent per patient prior to toxin detection. This prolonged period and need for repeated sample testing during a single admission implies the patient had more complex medical problems and therefore may be an indicator that diarrhoeal symptoms may not have initially been a result of *C. difficile* disease. Underlying medical disease and complex chronic medical conditions doubles the likelihood of colonisation with toxigenic *C. difficile* strains (Rexach *et al.*, 2006). High *C. difficile* prevalence has also been documented in hospitalised paediatric patients with inflammatory bowel disease (Pituch, 2009). Previous exposure with 3 or more antibiotics particularly within a 30day period increases the risk of severe CDI in the paediatric population (Kim *et al.*, 2012). Of these positive patients, 23% went on to be identified as toxin-positive following a negative sample, using 28 days as a cut-off between positive samples (range 28 to 104 days) on separate admissions. This suggests that just under one-quarter of toxin positive patients develop re-infection or re-colonisation with toxigenic *C. difficile* strains. Interestingly Rexach *et al.*, 2006 noted that paediatric in-patients were less likely to be colonised with toxigenic *C. difficile* strains than out-patients and Benson *et al.*, (2007) noted a significant increase in incidence of CDI in the out-patient setting. This sub-group of positive patients have risk factors for *C. difficile* acquisition including their recent hospital admission and therefore re-colonisation with *C. difficile* in the community leading to disease recurrence rather than re-infection from their original strain may be more common in the paediatric population.

Within General Medicine the apparent dramatic decrease in potential CDI rates seen from 2003 onwards may reflect a change in initial admission and treatment protocols as patients are now rapidly transferred to an appropriate sub-specialty following administration of initial treatment in acute services (Accident and Emergency, Acute Receiving Units and Combined Assessment Units). There was also a change in hospital administration policies for general medicine subsequent to relocation into new hospital premises in 2003 and introduction of the hospital at night scheme.

Approximately one-tenth of all toxin-positive patients were transferred through a minimum of two specialties when remaining positive for *C. difficile* toxins. The maximum number of inter-specialty transfers (and therefore wards) for an individual

CDI patient was six. This does not take into account the number of individual bed moves within the same ward itself. *C. difficile* spores contaminate the hospital environment and are more likely to be recovered from a room housing a CDI patient (Dubberke *et al.*, 2007) and can be recovered from the room for up to 28 to 40 days following discharge of a CDI patient (Verity et al 2001, Poxton *et al.*, 2001). Acquisition of CDI is more rapid occurring after 3.2 days for patients who share a room with a CDI patient, compared with 18.9 days compared to those patients who do not have direct or close contact (Keshava *et al.*, 2002). Therefore CDI spread secondary to transfer of patients through various specialties and thus wards is an important risk factor that infection control measures should also address.

Estimated costs over the study period for toxin testing kits alone were in the region of £126 500 (€143 000). On the basis of the most recent published European costs of nosocomial CDI published at the time of the initial analysis by Vonberg *et al.* (2008), the excess costs per CDI in-patient case was €7147. This was in accordance with the only published cost figures available for Great Britain from Wilcox *et al.* where extra costs were estimated at £4000 per CDI case. Without adjusting for inflation the potential excess costs for CDI patients over the study period, demonstrated in Figure 3.5, increased from approximately £3.5 million to £29 million.

Local *C. difficile* strains associated with CDI during this study period belong to ribotypes 001 and 106 (Mutlu *et al.*, 2007). Ribotypes 027 and now 078 (Goorhuis *et al.*, 2008) have been associated with increasing CDI rates and more severe disease, however, ribotype 027 has not been identified in this region during the period of the study. The incidence of potential CDI demonstrated locally by this study is similar to regions where ribotype 027 were prevalent. Control of CDI should not be solely focused on ribotype 027 and strategies to reduce CDI should consider the pathogenic potential of local strains.

This study is limited by review of potential rates of specialty-associated CDI based on appropriately tested toxin positive faecal samples rather than defined symptomatic disease. Additionally we have not been able to provide mortality rates and associated co-morbidity for the entire cohort. However, it was the first study from Scotland demonstrating CDI laboratory workload, recent CDI trends, maximal potential

specialty-dependent clinical CDI rates and provided valuable information on the burden of disease prior to mandatory data reporting, introduced in October 2006, and for age groups aged below 65 years, prior to 2009.

The incidence of patients identified with CDI despite rising markedly appears now to be decreasing. Not surprisingly the incidence has also been noted to increase with age, however, approximately one fifth of potential CDI cases occurred in those aged under 65 years. Medicine of the Elderly had a much lower incidence than several other specialties and therefore risk assessment of CDI development and containment should now also be targeted within other specialties including paediatrics.

With a reasonable proportion of identified CDI patients transferred through different specialties and the significant financial burden CDI imposes on healthcare institutions judicious application of infection control measures remains an important factor in preventing CDI transmission.

4. *Clostridium difficile* and colorectal surgery

4.1 Introduction

Clostridium difficile causes a spectrum of disease ranging from asymptomatic carriage, simple colitis, pseudomembranous colitis, fulminant colitis and death. The fulminant form of *Clostridium difficile* infection (CDI) includes the presence of hypotension, unremitting ileus, toxic megacolon and perforation. This extreme fulminant form of CDI has been found to develop in 3% to 10% of patients (Dallal *et al.*, 2002; Olivas *et al.*, 2010).

Surgical intervention is generally only required to treat severe CDI cases i.e. patients with fulminant CDI, with the indications for surgical intervention including toxic megacolon, perforation, peritonitis, progressive multi-organ dysfunction, vasopressor requirements and refractory sepsis due to a failure to respond to medical management. Surgical intervention is generally associated with high mortality rates ranging from 30% to 80% and therefore determining the optimal timing for surgical treatment is one of the greatest challenges associated with the management of fulminant CDI.

The most commonly performed surgical procedure for fulminant CDI is a subtotal colectomy (rectal sparring) with end ileostomy formation. Hemi-colectomies have also been reported with some success as a colonic sparing alternative. More recently Neal et al. (2011) describe a complete colonic sparring procedure for fulminant CDI treatment, where possible a loop ileostomy is formed laparoscopically, the colon is then lavaged with polyethylene glycol via the ileostomy, a catheter is left in the efferent limb of the loop ileostomy and vancomycin delivered through this to the colon for 10days.

This study used locally collected data to assess the potential impact on surgical services in particular colorectal surgery, within the adult in-patient population from 2000 to 2006 inclusive. Incidence rates from chapter 3 have been referred to for individual surgical specialties and therefore additionally include 2007 data. Transplant surgery (hepatic and renal) had to be excluded due to the combination local ward set-up with renal medicine. However, studies have found CDI to be an independent risk

factor for mortality in hospitalised liver transplant patients and found that patients in the early post-transplant period had the highest risk of developing CDI (Ali *et al.*, 2012; Albright *et al.*, 2007)

4.2 Results

4.2.1 *Clostridium difficile* in Surgery

The surgical specialties were assessed to determine the number and trends of CDI episodes occurring over the study period. As per previous, a new potential CDI episode was defined as "Only persons that have not been diagnosed with *Clostridium difficile*-associated disease within the previous 28 days are counted as new cases" (Materials and Methods section 2.1.3).

Colorectal surgery had the greatest number of CDI episodes over the study period, 399 episodes, with an exponential increase to 2003 and a further peak in 2006. Upper - gastrointestinal (UGI) surgery had the second highest number of episodes, 225 episodes, however an exponential increase was demonstrated from 2002 to 2004, again with a further peak in 2006. Following these in descending order the number of episodes per surgical specialty were Urology – 162 episodes, Orthopaedic and Trauma Surgery – 152 episodes, Cardiothoracic Surgery – 71 episodes, Vascular surgery – 64 episodes and other surgical specialties – 36 episodes (includes ENT – ear, nose and throat; Plastic surgery and Maxillo-facial surgery).

Colorectal, UGI and Urology surgical specialties demonstrated a decrease in number of CDI episodes from 2006 – 2007. Orthopaedic and Trauma surgery demonstrated a peak in 2005 and the remaining specialties have essentially seen a small but steady increase in the number of episodes over the latter years (Figure 4.1).

Paediatric surgery was considered separately with 19 CDI episodes diagnosed in 14 patients (aged under 18 years old) over the study period, one 14-year old patient had been admitted through the colorectal surgical service and one 13-year old patient through the UGI surgical service.

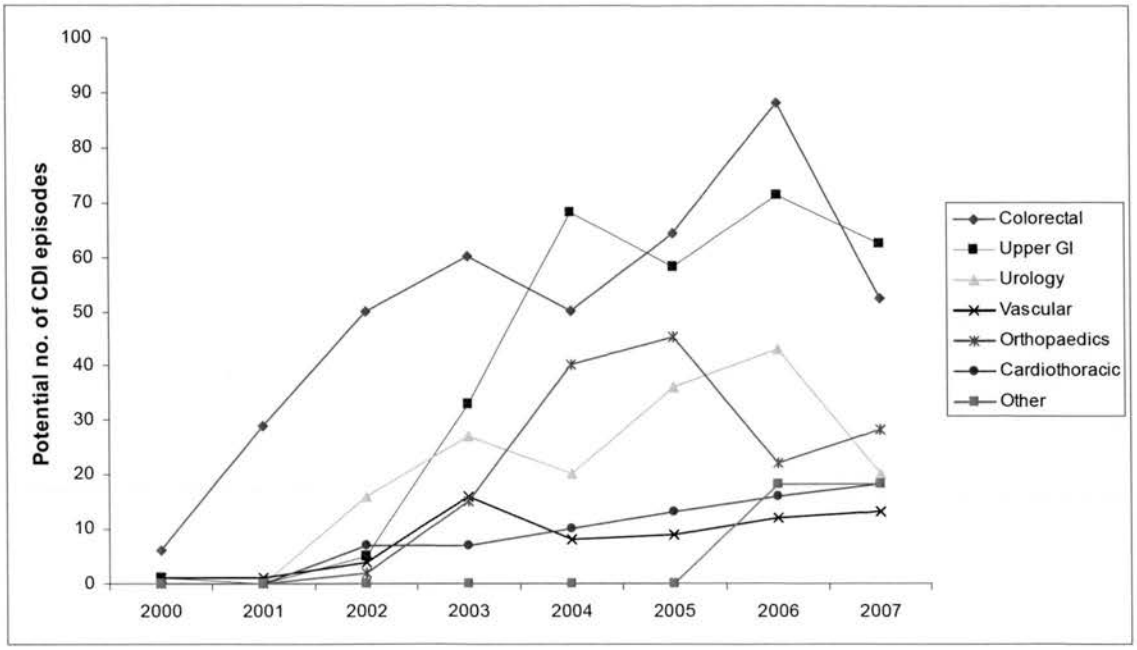


Figure 4.1 Trends in the number of CDI episodes per surgical specialty per year from 2000 to 2007.

4.2.2 *Clostridium difficile* impact on colorectal surgery

The total number of diagnosed toxin positive *C. difficile* adult in-patients, across all specialties, medicine and surgery, from 2000 to 2006 increased from 98 patients in 2000 to 1054 patients in 2006, with a total of 3895 patients over the study period. Of these 7.4% i.e. 288 patients were treated by the colorectal surgical service, with the number of toxin positive colorectal patients rising from 4 in 2000 to 76 in 2006.

Despite this rising trend in the number of patients identified as *C. difficile* toxin positive a similar continued increase was not seen in the number of patients diagnosed with pseudomembranous colitis with 90 patients diagnosed with PMC over the study period. These patients were diagnosed by histo-pathology from biopsy and colectomy specimens and identified from available endoscopic reporting. There was a peak in 2001 in the number of patients identified with PMC with a further peak demonstrated from 2004 to 2006.

Similarly during 2000 to 2006; the number of patients treated by colorectal surgery for fulminant CDI did not increase and a total of 23 patients underwent surgical intervention due to CDI (Figure 4.2).

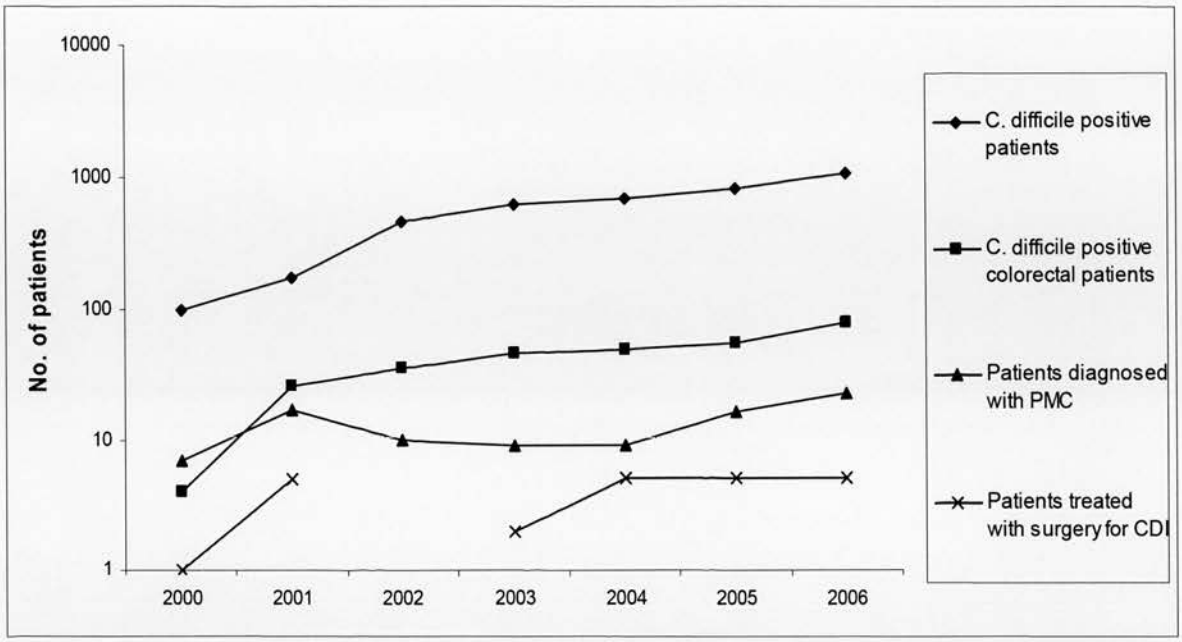


Figure 4.2 Comparison between the numbers of patients diagnosed with CDI, PMC and those patients who required surgical intervention for CDI, the number of patients has been plotted on a logarithmic scale due to the large difference in numbers.

Year	Total number of *C. difficile* toxin positive patients	*C. difficile* toxin positive colorectal patients	Number of patients diagnosed with pseudomembranous colitis	Number of patients treated with surgical intervention for CDI or fulminant PMC
2000	98	4 (4.1%)	7 (7.1%)	1
2001	175	26 (14.9%)	17 (9.7%)	5
2002	463	35 (7.6%)	10 (2.2%)	0
2003	612	45 (7.4%)	9 (1.5%)	2
2004	687	49 (7.2%)	9 (1.3%)	5
2005	806	53 (6.6%)	16 (2%)	5
2006	1054	76 (7.2%)	22 (2.1%)	5

Table 4.1 Tabulated data of figure 4.2. The proportion of *C. difficile* positive colorectal patients compared with the total number is given in brackets, as is the proportion of patients diagnosed with PMC compared with the total number.

4.2.3 Surgical Intervention for *Clostridium difficile* infection

Twenty-three patients underwent surgical intervention for CDI during the evaluated period, with pathological confirmation of *C. difficile* in their colectomy specimens. The median age of patients was 70 years (range 23 to 91 years), with 7 patients aged less than 65 years and 3 patients less than 45years. There was no particular gender difference with 11 female and 12 male patients (table 4.2).

Seven patients (30%) were direct emergency admissions to the surgical service from home. Three patients (13%) were current surgical in-patients who developed CDI during their admission. The remaining 13 patients (57%) were transferred from another specialty; UGI surgery (n=2), Vascular Surgery (n=2), Orthopaedics (n=3), Urology (n=1), Neurosurgery (n=1), Haematology (n=1), Gastrointestinal medicine (n=1), General Medicine (n=1) and Respiratory Medicine (n=1), following treatment for cholangitis, urinary tract infections, perinephric collections, pneumonia, leg ulcers and lymphoma; and following surgical procedures including femoral fixation, total hip replacements, below knee amputation, subarachnoid haemorrhage drainage and laparotomy for small bowel obstruction.

Twenty patients (87%) had taken antibiotics within two weeks prior to the development of CDI and had received broad-spectrum antibiotics which included second generation penicillins, cephalosporins, fluoroquinolones and clindamycin. In addition pipericillin and meropenem were also given as supplementary or replacement antibiotics in four cases. Fourteen of these 20 patients had received at least two of these antibiotics (Table 4.3).

The presenting abdominal symptoms and signs included abdominal pain in 21 patients (91.3%) this was localised to the right side in four patients and left side in two patients with the remainder experiencing generalised abdominal pain. Abdominal distension was present in 12 patients (52%) and nausea and vomiting in four patients. Diarrhoea was only present in 14 patients (61%). Localised or generalised peritonism on abdominal examination was present in 10 patients (43%) (Table 4.4). Haemodynamic parameters were adopted from Dallal *et al.* (2002) and Longo *et al.* (2004), seven patients (30%) were hypotensive with a systolic blood pressure of

<90mmHg, six patients (26%) were markedly tachycardic with a pulse >120 beats per minute, three patients were on Atenolol (beta-blocker) and remained normocardic pre-operatively. Pyrexia was not a common feature; with a median of 37.5°C (range 35.4°C to 38.9°C). Nine patients required vasopressor/inotropic support prior to surgery.

On laboratory blood tests, most patients (83%) demonstrated a leucocytosis with a neutrophilia, median 29.6 (range 0.4 to 77.1 x 10^9/l; neutrophils 0.02 to 71). Two patients were neutropaenic; one patient had received a recent cycle of chemotherapy for lymphoma, the other due to severe sepsis. Elevated platelets were also noted with a median of 412 x 10^9/l (range 10 to 617 x 10^9/l). Renal dysfunction was present in nine patients (39%) determined by a creatinine > 1.5 x baseline for the patient with a median creatinine of 96 (range 35 to 310 i.u.) (table 4.5). Only 12 patients (52%) had an arterial blood gas available on review of their notes with seven of these patients demonstrating a metabolic acidosis with abnormal hydrogen ions- H^+ ranging from 49 to 84. Hypoalbuminaemia was common pre-operatively with a median of 25 (range 10 to 35), a more profound hypoalbuminaemia however was noted post-operatively median 13 (range <10 to 23).

Only 12 (52%) patients had a *C. difficile* toxin positive faecal sample prior to surgery, eight patients had had a negative toxin result prior to surgery and three patients had no result available. On radiological imaging five patients had toxic megacolon demonstrated on plain radiograph; pneumoperitoneum was identified in one patient consistent with a viscus perforation. Seven patients underwent CT imaging of the abdomen and pelvis, in five of the seven patients a pancolitis (diffuse colonic thickening) was present, in one of the seven scanned patients segmental colitis of the right colon was present, and in one patient the CT scan was performed for a patient who had undergone a previous anterior resection with the scan revealing an anastomotic leak. A water soluble enema was performed in three patients with no obstructing lesions or leak seen; however some mucosal irregularity of the colon was noted on these studies. Five patients underwent a rigid or flexible sigmoidoscopy prior to surgery, and all these patients were found to have pseudomembranes.

Of the 23 patients only nine patients were thought to have a diagnosis of fulminant pseudomembranous *C. difficile* colitis just prior to surgical intervention. Three patients underwent a Hartmann's procedure. Two patients had undergone a sigmoid colectomy and anterior resection due to diverticulitis and a rectal cancer respectively during their admission; these patients went on to develop sepsis due to anastomotic dehiscence and underwent Hartmann's procedures; both their resection specimens demonstrated pseudomembranous colitis. A Hartmann's procedure was performed in another patient due to an intra-operative finding of a distal descending colon perforation with a thickened sigmoid colon and turbid free fluid; the pathology from this patient's specimen revealed PMC and B cell lymphoma.

Segmental colectomies were performed in a further five patients; four right hemicolectomies and a sigmoid colectomy. Of the four hemi-colectomies only one was performed for a query of *C. difficile* colitis intra-operatively; one was performed for an obstructing caecal mass with ischaemic appearing small bowel at the time of surgery, pathology revealed a Duke's C caecal cancer with pseudomembranes throughout both the colon and resected ileum. Of the other two; caecal wall thickening with a possible caecal mass were observed intra-operatively, the pathology specimens demonstrated pseudomembranous colitis only. The sigmoid colectomy was performed for presumed diverticulitis with a thickened sigmoid colon noted intra-operatively; the pathology demonstrated pseudomembranous colitis and diverticular disease.

Sub-total colectomy and end ileostomy formation with sparing of a rectal stump was performed in the majority of patients (n=15; 65%). This procedure was performed in eight patients thought to have a diagnosis of fulminant CDI prior to the procedure or at the time of surgery with one patient, who had received chemotherapeutic agents diagnosed with typhlitis. Of the remaining seven patients the procedure was thought to be performed for ischaemic colitis (n=3), Crohn's colitis (n=1), megacolon due to a chronic amotile large bowel with secondary sepsis (n=1) and toxic megacolon due to an unknown cause (n=2). Perforation was identified in three patients intra-operatively and large volume ascites in six patients. Thickened oedematous dilated colon was identified in all patients in this cohort with a pancolitis in 13 of the 15 patients, oedema of the right colon with cyanotic appearance of the left colon in 1 patient and

involvement of the small bowel in addition to abnormal ascending and transverse colon in 1 patient. Pseudomembranous colitis was found in all the pathological specimens, with associated Crohn's disease in one patient and pseudomembranes with ulceration and necrosis within the 50cm of additional resected terminal ileum in one patient.

Post-operative complications of the entire operative cohort included lower respiratory tract infections and pulmonary insufficiency (n=8), congestive cardiac failure (n=1), post-operative intra-abdominal bleed (n=1) and rectal stump bleed (n=1), upper GI bleed due to a bleeding duodenal ulcer (n=1), fractured neck of femur following a fall (n=1) and large bowel obstruction with repeat laparotomy required in an Hartmann's procedure patient as the colon had twisted on its mesentery (n=1). Overall the post-operative morbidity was 70% (16 of 23 patients).

Six patients (26%) died in the 30-day post-operative period (mean 10 days (range 2 to 21 days). Three out of 15 patients (20%) died in the sub-total colectomy and end ileostomy cohort, and three out of eight patients (37.5%) died in the segmental colectomy and Hartmann's procedure cohort. The median ITU admission of the survivors (17 patients) was 2 days (range 0 to 41 days) and the median HDU admission 6 days (range 1 to 10 days), with an overall hospitalisation or time to discharge from the surgical ward of 18 days (range 9 to 74 days) (table 4.6). Four of the 17 survivors (24%) underwent reversal of their stoma.

There was no statistical significance in post-operative mortality or median hospitalisation between the two surgical cohorts (sub-total colectomy and end ileostomy versus segmental colonic resection). Neither was there any statistical significance in age, pre-operative white cell count and creatinine parameters between the survival and mortality groups (Tables 4.6 and 4.7).

Patient Demographics	Data
Median age (years)	70 (23-91)
Male: Female ratio	12 : 11
Symptoms developed at home	7 / 23 (30%)
Symptoms developed in hospital	16 / 23 (70%); 13 transfers to Colorectal Surgery.

Table 4.2 Data for patient demographics provided as numbers or ranges with percentages in brackets.

Major risk factors	No. of patients
Antibiotic use within 2 weeks of CDI symptoms	20/23 (87%)
Recent intra-abdominal surgery	3/23 (13%)
Recent surgery out with the abdomen	5/23 (22%)
Chemotherapy	1/23 (4%)
Other immunosuppressive agents including steroids	4/23 (17.3%)

Table 4.3 The number of patients compared with the total with specific risk factors for CDI development with the proportion of patients in brackets.

Presenting symptoms	No. of patients
Diarrhoea	14/23 (61%)
Abdominal Pain	21/23 (81%)
Abdominal Distension	12/23 (52%)
Peritonitis/Surgical abdomen	10/23 (43%)

Table 4.4 The number of patients with the main presenting symptoms, the proportion of patients is given in brackets.

Laboratory and diagnostic studies	Data
Leucocytosis; median white blood cell count	19/23 (83%); median 29.6 (0.4 to 77.1 x 10^9/l)
Renal dysfunction; median creatinine μmol/l	9/23 (39%); 96 (range 35 to 310)
C. difficile toxin positive faecal sample	12/23 (52%)
Sigmoidoscopy	5/23 (22%)
Toxic Megacolon on plain radiographs	5/23 (22%)
Colitis on Computerised Tomography imaging	6/23 (26%)

Table 4.5 Data for laboratory and diagnostic studies performed pre-operatively with the proportion of patients in brackets and median ranges where appropriate given.

Surgical procedure and post-operative variable	Subtotal colectomy and end ileostomy (n=15)	Segmental colectomy (n=8)	p value
Procedure	As above	Right hemicolectomy n=4 Sigmoid colectomy n=1 Hartmann's Procedure n=3	-
Post-operative surgical morbidity	10/15 (67%)	6/8 (75%)	-
30-day post-operative mortality	3/15 (20%)	3/8 (37.5%)	0.66
Median hospitalisation / time to discharge of survivors (days)	20 days (12-46)	15 days (9-74)	0.34

Table 4.6 Surgical procedure and post-operative variables for each surgical cohort with the proportion of patients for each cohort in brackets and the p value provided.

Variable	Survivors (n=17)	Mortality (n=6)	P value
Mean age (years)	77.7 years (23 – 85)	75 years (65 – 91)	0.078
Mean white blood cell count x10^9/l	27.7 (0.4 – 54.4)	24.7 (5.3 -77.1)	0.66
Creatinine μmol/l	113 (35 – 282)	137 (77 – 310)	0.56
Pre-operative inotropic support	8/17 (47%)	3/6 (50%)	-

Table 4.7 Comparison of variables in the post-operative survivors and mortality groups, with proportion of patients or ranges given in brackets for each variable, and the p value between the survivors and mortality cohorts also provided.

4.2 Discussion

Although a relative plateau in incidence was seen in General Surgery from 2002 to 2006, as described in Chapter 3, the figures combined both colorectal and UGI surgical service figures. On separating the two services, review of the number of episodes demonstrated peaks and troughs within these two surgical specialties allowing equilibrium when calculating the incidence for general surgery as a whole. Often colorectal and UGI surgery are either grouped together or clear delineation between the two groups is not clarified when general surgery CDI figures are quoted. With these two surgical specialties separate entities in most academic/teaching institutions with differing case workloads, patient cohorts and fulminant CDI generally treated by colorectal surgery, the distinction is important. In this study episodes of CDI were more common in the colorectal rather than UGI surgery cohort; whilst this may seem intuitive few studies have commented on the UGI surgery population. Yasunaga et al. (2012) reviewed patients undergoing surgical intervention for cancer, from 2007 to 2010 and found a higher occurrence of CDI in their colorectal surgery group compared with the gastrectomy and oesophagectomy group. Zerey et al. (2007) reviewed surgical patients from 1999 to 2003, and determined colectomy or small bowel resections were associated with the highest risk of CDI compared with gastrectomy and that patients undergoing a cholecystectomy or appendicectomy had the lowest risk.

The incidence of potential CDI in Urology, was 1.37/1000 OBD (Chapter 3). This was much higher than observed by Hossain *et al.* (2008), who reported an incidence of 0.66 cases /1000 OBD, they also found no significant increase in *C. difficile* incidence between 2000 to 2005 with a range of 3 to 13 cases per year. Conversely locally, the number of CDI episodes in Urology has overall gradually been increasing to 2006, with the number of episodes varying from 16 to 43 per year. In part this may be due to the increase use of fluoroquinolones; in 2006 the European Urology Association guidelines recommended the use of fluoroquinolones in the treatment of simple cystitis, pyelonephritis, epididymo-orchitis, prostatitis and uro-sepsis (Patel, 2007).

The number of episodes in Orthopaedics and Trauma Surgery gradually rose from two episodes in 2002 to a peak of 45 episodes in 2005 with a subsequent decline and an overall incidence of 0.62/1000 OBD. Specific data on trends of CDI in this specialty were not available for comparison. Kurd *et al.* (2008) reported on 16 cases of CDI from 2001 to 2006, in patients who had undergone total joint arthroplasty, with an incidence of 0.16% and no significant increase in CDI over their study period. One of the tertiary hospitals used in this study has one of the largest Accident and Emergency departments in Europe, consequently there is a large referral of trauma patients, Al-Obaydi *et al.* (2010) found the rate of CDI to be 8-fold higher in trauma than elective patients. Therefore the rise in numbers may reflect trauma rather than elective admission related CDI within this specialty, at a time where the overall incidence in the region was increasing.

Cardiothoracic Surgery has an overall low potential episode rate (0.95/1000 OBD) accounting for only 6% of all CDI episodes in the surgical specialties. However it has observed a small but gradual increase in the number of CDI episodes. This may reflect an increasing age and co-morbidity population undergoing cardiac surgery. Musa *et al.* (2011) reviewed cardiothoracic ITU admissions from 2003 to 2008 and found CDI was rarely acquired in cardiothoracic ITU and was more common in post-operative cardiac surgery rather than thoracic surgery patients. Crabtree *et al.* (2007) also noted an overall low incidence of 0.79% of CDI in cardiac surgery patients between 1997 and 2004 with a peak incidence in 2003.

Vascular surgery has previously been quoted as having high CDI rates of 6-9% along with general surgery (Bradbury & Barrett, 1997). In this study vascular surgery had one of the lowest number of CDI episodes when compared with the other specialties, with a peak in 2003. Previous high rates were attributed to the increased use of clindamycin in this specialty to treat infections associated with peripheral vascular and venous disease. However as this was an early surgical specialty to describe *C. difficile* related disease, this may have resulted in earlier increased awareness. Ratnayake *et al.* (2011) reported an outbreak of CDI of ribotype 106 with high-level of clindamycin resistance in nine patients on a vascular surgery unit within East Scotland, therefore high-lighting that even in specialties with now low rates of CDI continued attentiveness to infection control is required.

Of note the main general surgical specialties, namely colorectal and UGI surgery, were the only specialties that had faecal samples documented for *C. difficile* testing by the enteric laboratory with positive results from 2000 to 2002. Vascular surgery also had documented samples sent for *C. difficile* testing but no positively identified CDI samples. However, all the remaining surgical specialties had no documented samples requested for *C. difficile* testing until 2002. This may represent a change in sample testing and recording by the enteric laboratory from 2000 to 2002, samples may have been sent without the specialty to which they were attributed known and therefore categorised in an unknown cohort. General increased awareness of *C. difficile* particularly from 2002 onwards may have also resulted in surgical specialties recognising and testing for CDI. The decline in the number of episodes between 2006 and 2007, in the surgical specialties with the greatest number of CDI episodes, follows the overall general downwards trend seen locally as described in Chapter 3.

Nineteen episodes of CDI were identified in the paediatric surgical population accounting for 20.8% of all paediatric associated episodes during the study; increasing from one episode in 2001 to seven episodes in 2006. Chen *et al.* (2012) found the paediatric incidence of CDI doubled from 2002 to 2008, and was identified in primarily immunosuppressed patients, none of whom required surgical intervention for treatment. Kim *et al.* (2012) similarly determined that although severe CDI accounted for a significant proportion of their paediatric surgical cohort with CDI, complications including fulminant colitis were uncommon.

Despite the increased number of patients across all specialties found to be toxin positive from 2000 to 2006, a similar continued increase was not demonstrated in the number of patients diagnosed with pseudomembranous colitis or the number of patients that underwent surgery for fulminant CDI. This is in contrast to other studies which have stated an increase in the number of patients requiring surgical intervention for CDI (Longo *et al.*, 2004, Koss *et al.*, 2006; Lesperance *et al.*, 2011). Although patients are at risk of acquiring CDI within the specialty of colorectal surgery itself, patients are also referred from other specialties for surgical intervention due to severe fulminant *C. difficile* colitis and typhlitis. The plateau in surgical intervention patients may suggest that patients were not being referred for surgical review, patients were

not suitable for surgery or disease severity did not warrant surgical intervention. Most studies demonstrating an increased incidence of CDI in General Surgery and the number of patients requiring surgical intervention were from areas where ribotype 027 was prevalent. This hypervirulent strain was not endemic to the area during the period of the study and therefore the extent of more extreme forms of CDI may not have been seen locally.

The proportion of toxin positive colorectal patients compared with the total number of toxin positive patients per year did not appreciably change (approx 7% - 8% from 2002 – 2006) with the exception of 2001. Therefore the increase in toxin-positive patients in colorectal surgery is proportionate to the increase demonstrated locally and again suggests that few patients were likely referred, needed or were suitable for colorectal surgical management from other specialties. However, as the number of individual toxin-positive colorectal surgical patients has markedly increased this does impact on the overall burden of CDI within the specialty with implications for treatment and infection control.

The numbers of patients with pseudomembranous colitis are likely to be under-estimated, as not all patients with pseudomembranous colitis will have undergone a flexible sigmoidoscopy/colonoscopy or had biopsy specimens sent. Even with this limitation, the proportion of PMC patients identified from 2002 to 2006 compared to the total number remained between 1.3% and 2.2% and again may relate to absence of ribotype 027 locally. Of interest the proportion of PMC patients in 2000 and 2001 was much higher. This is more likely a reflection of decreased awareness of *C. difficile* in many specialties as also seen in some surgical specialties during these years and therefore a decreased number of toxin positive CDI patients diagnosed in 2000 and 2001. However in 2001, five patients were surgically treated for fulminant CDI similar to 2004 to 2006 where the number of overall toxin positive patients increased four to eight fold.

Severe CDI, PMC and fulminant colitis, was present in 2.3% of this study population and the fulminant form of CDI has been found to develop in 3% to 8% of patients in other studies (Dallal *et al.*, 2002; Olivas *et al.*, 2010). However not all patients with fulminant CDI require surgery. Only 0.6% of the total population of toxin positive *C.*

difficile patients underwent surgical intervention as a result of CDI during the period of this study. This is similar to Koss *et al.* (2006) who found 0.4% of their studied CDI population underwent colectomy. However both these figures are lower than other studies that have quoted figures of 1.6% to 3.7% (Dallal *et al.* 2002; Al-Abed *et al.*, 2010). Similarly this study found that 25.5% of patients with severe CDI went on to require surgical management, which is similar to figures quoted by Bhangu *et al.*, (2012) who performed a systematic review of patients requiring surgery for CDI and found 29.9% of patients with severe CDI required surgery with a range of 2.2% to 86% for individual studies.

The median age of the 23 patients who underwent surgical intervention for CDI during the study was 70 years (range 23 to 91 years). This median age is similar to other studies ranging from 64 years to 71 years (Koss et al 2006; Gash et al 2010). With 7 patients aged less than 65 years and 3 less than 45 years, this is further evidence that CDI and its complications are not purely related to an elderly population.

Most studies of surgery for fulminant CDI documented the majority of patients were transferred from other specialties as with this study. Of interest are the emergency admissions from home requiring emergency surgery for severe CDI, in this study accounting for 30% of patients, unlike hospitalised in-patients a good history with known risk factors for CDI are often not available. Diarrhoea was not present in 39% of patients at presentation, as a result of severe colonic dysfunction and ileus associated with fulminant colitis. Similarly 48% of patients did not have a *C. difficile* toxin positive faecal sample result available prior to surgery and in this cohort most were the emergency admissions. Therefore high clinical suspicion is required in these patients with probable community acquired CDI particularly if a history of prior antibiotic exposure can be elicited.

Eighty-seven percent of the patients who received surgical intervention had received antibiotics within two weeks prior to the development of their fulminant CDI symptoms and had received broad-spectrum antibiotics including second generation penicillins, cephalosporins, fluoroquinolones and clindamycin and 61% had received two or more antibiotics. Since this study was performed there has been a change in

local antibiotic prescribing with greater stewardship. In colorectal surgery the commonly used combination of ceftriaxone and metronidazole, during this study, for the treatment of intra-abdominal sepsis and prophylaxis within an hour of major intra-abdominal surgery, has changed with cephalosporins being replaced by other alternatives such as gentamicin (Lothian prescribing guidelines).

Independent risk factors for mortality for patients undergoing surgical intervention for CDI have been reported and these include a high pre-operative lactate, a high pre-operative leucocytosis, a low pre-operative albumin, pre-operative vasopressor requirements, development of end-organ failure with pre-operative renal failure and a need for pre-operative intubation and ventilation, advanced age, altered mental status and immunosuppression (Dallal *et al.*, 2002; Byrn *et al.*, 2008; Lamontagne *et al.*, 2007; Pepin *et al.*, 2009; Perera *et al.*, 2010). In this study several of the reported independent risk factors were noted; 83% of patients demonstrated a marked leucocytosis with a neutrophilia and 8.7% were neutropaenic, renal dysfunction was present in 39%, hypoalbuminaemia was present in all patients pre-operatively with a profound hypoalbuminaemia post-operatively, 21.7% were receiving immunosuppressive agents, 30% of patients were hypotensive and 47.8% required pre-operative vasopressor support. No patients required pre-operative intubation out-with theatre. However on further review between the cohort of patients that survived and those that died post-operatively there was no significant statistical difference between the groups for the parameters of age, white cell count, pre-operative renal dysfunction or creatinine levels. We were unable to statistically analyse the need for vasopressor support due to an absence of complete data in the clinical notes. Similarly in a systematic review and meta-analysis performed by Bhangu *et al.*, (2012) of patients requiring surgery for CDI, a leucocytosis of 20 x 10^9/l or more and pre-existing renal failure were not significant predictors of death following surgery and there was insufficient data on albumin and lactate levels to meta-analyse. They did find that pre-operative intubation, shock requiring vasopressors and multi-organ failure were identifiable predictors of post-operative death.

A number of severity scoring systems for CDI have been developed to predict mortality including usage of the Charlson co-morbidity score and the APACHE II (Acute Physiology and Chronic Health Evaluation II) score. However following

critical review all severity scoring systems were unable to predict the need for surgical intervention or predict survivability post-operatively (Pepin *et al.*, 2009; Carchman *et al.* 2012; Bhangu *et al.,* 2012)

Pre-operative radiological imaging was performed in 69.5% of patients ranging from plain radiographs to water soluble enema studies to CT imaging. In this study seven patients underwent CT imaging of the abdomen and pelvis and a pancolitis was present in five patients, segmental colitis in one patient and an anastomotic leak in one patient following previous surgery. Since the period of this study water soluble enemas are also being superseded by CT for the acute abdomen, and studies have found a benefit to CT scanning in diagnosing and evaluating complicated CDI with implications for therapy guidance (Valiquette *et al.*, 2009; Kirkpatrick *et al.*, 2001). Longo *et al.*, 2004 found CT to be the most sensitive indicator of assessing CDI and severe colonic inflammation in their CDI operative series. The value of the plain abdominal radiograph however should not be neglected as toxic megacolon was demonstrated in 21.7% of patients negating the need for CT and nephrotoxic intravenous contrast agents. However a CT scan or flexible sigmoidoscopy prior to surgery is of benefit when diagnosis is in doubt or clear indications for surgery are absent, particularly in the absence of a *C. difficile* toxin positive faecal sample result.

Three patients (13%) developed CDI during their in-patient admission following previous colorectal surgery of which two were elective patients and one an emergency patient. Two of these patients were found to have anastomotic leaks, likely as a result of their severe CDI disease, and went on to have Hartmann's procedures performed. Two further patients were found to have cancers, lymphoma and a Duke's C caecal cancer in there segmental resection specimens. Lesperance *et al.* (2011) reviewed a large cohort of patients who had undergone colonic surgery for causes unrelated to CDI and found that Caucasian race and a non-elective admission was predictive of CDI development during hospital stay. (The ethnicity of patients had not been recorded in our study). Interestingly they also found that diagnosis of a colonic malignancy and diverticulitis were negative predictors of CDI.

Small bowel *C. difficile* enteritis is rare and Kim *et al.* (2011) identified only 58 cases in the published literature from 1980 to 2010. Of these pooled cases 82% had had

previous GI surgery primarily a total colectomy and end ileostomy formation for non-CDI causes. However in the two cases of small bowel CDI enteritis found in the surgical cohort in this study neither patient had undergone a previous colonic resection. One patient was aged 91 and underwent a right hemicolectomy with increased terminal ileal resection, the other was aged 42 and underwent a total colectomy with a 50cm resection of the terminal ileum. Pseudomembranes were found throughout these resected specimens. Similarly Hayetian *et al.* (2006) reported a case of CDI involving the colon and distal ileum with perforation of the ileum found intra-operatively. Therefore in patients with fulminant CDI with CDI enteritis intra-operatively how much does the enteritis contribute to the extremity of disease and does the entire small bowel with macroscopic change need to be resected?

Sub-total colectomy and end ileostomy formation with sparing of a rectal stump was performed in 65% of the surgical intervention cohort and segmental colectomies in 35% comprising four right hemicolectomies, a sigmoid colectomy and three Hartmann's procedures. Overall the post-operative morbidity was 70% (16 of 23 patients) involving 67% of patients in the sub-total colectomy and end-ileostomy cohort and 75% of patients in the segmental colectomy cohort. The post-operative mortality was 26%; three out of 15 patients (20%) in the sub-total colectomy and end ileostomy cohort died, and three out of eight patients (37.5%) in the segmental colectomy cohort died.

Mortality associated with CDI generally is approx 6% (Karas *et al.*, 2010), however mortality associated with fulminant CDI where surgical intervention was performed has always been high varying from 19% to 64% (Neal *et al.*, 2011; Trudel *et al.*, 1995). A post-operative mortality rate of 26% was found in this study, and although it was towards the lower end of reported mortality figures it remains high. Despite increased CDI awareness and disease recognition mortality rates have not significantly changed over the past two decades. There has been much debate over the years about the type of surgery which should be performed for fulminant CDI. Traditionally subtotal colectomy with end-ileostomy formation has been recommended rather than a segmental resection, which was thought to be associated with higher mortality. However there was no significant difference between the two surgical cohorts in this study with many studies also finding equivocal survivability

rates in patients who undergo a segmental colectomy for CDI rather than a total colectomy and end ileostomy. (Dallal *et al.*, 2002; Longo *et al.*, 2004; Pepin *et al.*, 2009). Hemicolectomies/ segmental resections have been associated with 15.9% re-operation rates for CDI (Bhangu *et al.*, 2012), no patients, in particular the survivors, of the segmental colectomy cohort in this study required a repeat laparotomy due to CDI. The lowest mortality rate of 19% was reported in a study by Neal *et al.*, (2011), and they performed a diverting loop ileostomy with colonic lavage as an alternative to colectomy to treat complicated fulminant CDI, (this procedure is described in the introduction for this chapter). The biological rationale on which their procedure is based, is that by creating a loop ileostomy the faecal stream is interrupted thus depriving the colonic bacteria of nutrition, mechanical lavage through the efferent limb removes a proportion of the bacteria and surface toxins that induce the local inflammatory and cytotoxic response on the colonic mucosa and administration of vancomycin through a catheter left in situ within the efferent limb of the ileostomy should continue to treat the disease allowing the inflammatory process to settle. Oral metronidazole is also continued. From their cohort of 42 patients, three required colectomy for abdominal compartment syndrome. They compared their cohort of patients to their colectomy and end ileostomy cohort from 2009 and found a significant reduction in mortality of 19% compared with 50% previously. Their study certainly merits consideration for surgical therapeutic intervention in the future.

Early surgical referral is hampered by the absence of a validated diagnostic tool that can help predict at which stage a patient with CDI will benefit from surgical intervention. However the benefit of early surgical referral from other specialties should be highlighted. Studies have shown that colectomy for severe fulminant colitis prior to acute respiratory and acute renal failure in those aged greater than 65 years is associated with decreased mortality (Seder *et al.*, 2009). A more aggressive approach with early colectomy for those patients who fail initial treatment with antibiotics prior to the development of multi-organ failure has also shown decreased mortality rates (Jabar *et al.*, 2008; Sailhamer *et al.*, 2009). Therefore surgical management of CDI in patients who do not respond quickly to antibiotics may become a life-saving rather than a desperate procedure. With 43% of patients in this study presenting with localised or generalised peritonism on abdominal examination including those

patients transferred from other specialties it can be postulated that in these cases surgical referral was occurring much later than desired.

5. Identification of *Clostridium difficile* in colorectal surgical patients.

5.1 Introduction

Awareness of CDI has dramatically increased over the last decade with the first step to diagnosis being the recognition of clinical disease. Following clinical assessment and suspicion of symptomatic CDI, the diagnosis of CDI hinges on laboratory testing of faecal samples. Therefore rapid and accurate diagnosis of CDI is mandatory to individual patient treatment and infection control procedures.

Only toxigenic *C. difficile* strains cause disease and subsequently *C. difficile* toxin detection was the first diagnostic method employed and in a different guise remains the mainstay of hospital detection today. The cell cytotoxic neutralisation assay is still regarded as the gold standard for laboratory confirmation of *C. difficile* (Whittier *et al.*, 1993). The method uses detection of cytopathic effects in cell culture that is neutralised by antibodies to *C. difficile* or *C. sordellii* cytotoxins. These cell cultures are then analysed microscopically at 24 and 48 hours. This method, therefore, is time-consuming and needs availability of tissue cultures and a skilled workforce. The identification of toxins progressed further into the development of enzyme immunoassays (EIA) for toxin A or B or A&B detection kits which can detect toxins directly from faecal samples. These assays are quick, easy and relatively inexpensive to perform allowing the high turnover required with increasing numbers of faecal samples sent for testing. Subsequently commercially produced EIA toxin kits have been adopted as the diagnostic procedure of choice for CDI by hospital enteric laboratories. These kits however have been reported to have limited sensitivity and specificity (Crobach *et al.*, 2009).

Toxigenic culture is a further reference standard. The method relies on the anaerobic culture of *C. difficile* from faecal samples grown on media specific plates for at least 48 hours and the identification of typical colonies. This method identifies both toxigenic and non-toxigenic strains of *C. difficile*; therefore further assessment of identified colonies for in vitro toxin production is required. This method, as with cell

cytotoxin neutralisation assay is laborious, and again the method is used in an investigatory rather than clinical capacity.

Other newer commonly used hospital diagnostic laboratory tests now include EIA for the enzyme glutamate dehydrogenase. This enzyme is produced by both toxigenic and non-toxigenic *C. difficile* strains and therefore while this test identifies the presence of *C. difficile* it cannot differentiate between toxin and non-toxin producing strains. However, it has an extremely high negative predictive value (99%), and is useful for reporting *C. difficile* negative patients.

Another development is the application of real-time polymerase chain reaction (RT-PCR), this is a nucleic acid amplification method that can be used to detect toxin genes directly from faecal samples. It detects the presence of targeted genes; a conserved region of *tcdB*, which encodes toxin B production, or *tcdC*, the main negative regulator of toxin A and B production.

During the period of this study only commercially manufactured ELISA toxin kits, Tox A/B II™ (Techlab), were used as the diagnostic identification modality for CDI by the Lothian enteric laboratory (EL).

The aim of this study was to determine the proportion of faecal samples and hence patients not identified as toxin positive by standard enteric laboratory testing, using the reference standard of toxigenic culture for comparison. Further aims of the study were to determine asymptomatic carrier rates and to identify *C. difficile* in colorectal surgical in-patients and hence determine the specific burden of *C. difficile* in this patient cohort and to provide local epidemiological data from *C. difficile* isolates within this specialty.

There were two study periods; from June 2007 to November 2007 for recruitment of out-patient asymptomatic carriers, and from November 2007 to January 2009 inclusive for weekly reclamation of enteric laboratory submitted samples testing, with materials and methods as described in Chapter 2.

5.2 Results

5.2.1 Asymptomatic carrier rate in the out-patient surgical population.

Ninety-eight patients were recruited from patients attending the out-patient colorectal surgical clinic, attending for day case procedures or elective patients admitted for in-patient colorectal surgical procedures. Only those patients who had not been hospitalised or in a long-term care facility within the last six months and did not have symptoms of diarrhoea were recruited. Faecal samples were collected at the surgical out-patient clinic, or within hours (1-4 hours) of admission for day-case or in-patient procedures, therefore faecal samples were all representative of an out-patient cohort.

Six patients of 98 were culture positive for *C. difficile* and all were toxin producing *C. difficile* strains. Therefore the asymptomatic carrier rate from this cohort is 6.1% for toxigenic *C. difficile*.

Four of the six patients' toxigenic *C. difficile* strains were of ribotype 001, the other two could not be determined.

Two of these six asymptomatic carriers who were later admitted for surgical operative procedures went on to develop symptomatic CDI as in-patients.

5.2.2 Identification of *Clostridium difficile* in colorectal surgical in-patients.

Six hundred and thirty-two faecal samples from 483 adult colorectal surgical in-patients were reclaimed over the study period from the enteric laboratory for toxigenic culture.

The enteric laboratory (EL) found 61 (9.7%) of 632 faecal samples to be toxin positive. Of the remaining 571 faecal samples tested, 505 samples (79.9%) were toxin negative. Sixty-one faecal samples (9.7%) were not tested for three reasons; patients from whom they were submitted had already had a *C. difficile* toxin positive result in the last 28 days, duplicate sample requesting resulted in repeated samples being sent

on the same day for the same patient or the faecal samples were non-diarrhoeal (classified as ≤ 4 on the Bristol stool chart). Four samples were for enteric culture for other causes not related to CDI, and had inadvertently been sent for toxin testing.

On toxigenic culture of all the 632 faecal samples submitted, a further 72 faecal samples of the 505 EL-negative samples were culture positive for *C. difficile*, of these further identified 72 samples; 68 samples were toxigenic culture positive (i.e. toxin producing strains) therefore a further 10.8% of all submitted faecal samples were not identified as toxin positive by EIA testing alone.

Of the 61 EL toxin positive faecal samples; 47 (77%) faecal samples were also toxigenic culture positive on primary culture, with one EL toxin positive faecal sample only identified as toxigenic culture positive on a second re-culture. Fourteen samples found to be EL toxin positive were toxigenic culture negative on triplicate culture assessment; these faecal samples were also treated with 50% alcohol shock prior to tertiary re-culture to reduce bacterial overgrowth from other faecal flora.

From the sub-set of 61 not tested EL faecal samples, 14 of the samples not tested were toxigenic culture positive; including seven non-diarrhoeal samples. Six of these 14 samples were from patients previously toxin positive within a 28 day period and remained positive (range 1 to 24 days) with toxigenic culture. One of these fourteen toxigenic culture positive EL not-tested samples was a duplicate sample. Seven of the EL not-tested faecal samples from patients with previous toxin positive results in the last 28days were culture negative.

Overall 133 faecal samples from 102 colorectal in-patients were found to be culture positive and 129 were toxigenic culture positive from 99 colorectal in-patients, with 58 patients not identified as toxin positive by the enteric laboratory at the time the faecal samples were sent, over the period of the study.

Faecal samples variable	Enteric laboratory (n=632)	Toxigenic culture (n=632)
C. difficile positive samples	61	133
Toxin positive samples	61	129
Negative samples and not tested samples	566	499
Not tested samples	61	0
EL toxin negative - culture positive samples	-	72
EL toxin positive - culture negative samples	-	14

Table 5.1 Distribution of the number of faecal samples in each category as assessed in the enteric hospital diagnostic laboratory (EL) by toxin EIA kits or in the research laboratory with toxigenic culture.

5.2.3 Potential asymptomatic *Clostridium difficile* colorectal surgery in-patient carrier rate.

On reviewing all toxigenic culture positive samples 10 (13%) of the 68 toxigenic culture positive - EL toxin negative samples were grade 4 or less on the Bristol stool chart, all others were diarrhoeal. Seven of EL not-tested non-diarrhoeal samples were toxigenic culture positive and 4 of the 47 EL toxin positive and toxigenic culture positive faecal samples were non-diarrhoeal. These 21 toxigenic culture positive non-diarrhoeal stool samples were reclaimed from 19 patients (3.9%) of the 483 tested patients. On review of these patients' available clinical details there was no definitive evidence of CDI within the one month before and after the faecal sample submission date; this therefore suggests an in-patient carrier rate of 3.9%.

5.2.4 Characterisation of *Clostridium difficile* isolates obtained by toxigenic culture.

Of the 133 culture positive faecal samples, only one *C. difficile* colony from each sample was stored and used for analysis. Three toxigenic culture positive samples could not be cultured from their Robertson's cooked meat broth stores during the period of MIC antibiotic susceptibility testing and the period when cultures were being sent to the Health Protection Agency, Colindale for typing and therefore have not been included in this part of the study.

Over the entire study period ribotype 001 was the commonest ribotype found in the colorectal surgical in-patient faecal isolates, with 61 of the 130 isolates (46.9%) identified as ribotype 001. Ribotype 012 (6.9%) was the next commonest found in 9 of the 130 isolates, followed by ribotypes 015 (4.6%), 020 (3.8%) and 078 (3%) (Table 5.2 and figure 5.1). A ribotype was not determined in 21 of the 130 isolates (16.2%). Further ribotypes 003, 005, 010, 011, 014, 018, 026, 031, 039, 056, 103, 106 and 140 were identified in the remaining isolates.

All ribotype isolates were sensitive to metronidazole. Two isolates showed variable results to metronidazole with triplicate MIC dilution agar testing however with the E-test method both isolates were found to be sensitive to metronidazole. An MIC of 4μg/ml was repeatedly demonstrated in 6 isolates (4.6%) to vancomycin, denoting these isolates with intermediate resistance to vancomycin; the remaining 124 isolates were all sensitive to vancomycin. Intermediate vancomycin resistance was found in both the 103 isolates and in one of each of the following ribotype isolates 001, 003, 020 and 106.

The 61 isolates identified as ribotype 001 demonstrated variable resistance to ceftriaxone and ciprofloxacin. Thirty of the 61 ribotype 001 isolates showed intermediate resistance to ceftriaxone and 28 of these 30 isolates were resistant to ciprofloxacin, 22 isolates were resistant to ceftriaxone and again with the exception of one isolate were resistant to ciprofloxacin, and the remaining nine ribotype 001 isolates were sensitive to ceftriaxone with ciprofloxacin resistance present in only four of these isolates. Resistance to ciprofloxacin was therefore common in the ribotype 001 isolates with resistance to ciprofloxacin demonstrated in 93% of these isolates.

In the isolates with the next most common ribotype 012, all these isolates were sensitive to metronidazole and vancomycin, with ceftriaxone resistance found in 4 isolates and ciprofloxacin resistance in 3 isolates.

Overall ceftriaxone resistance was found in 35 of 130 isolates (26.9%) and intermediate ceftriaxone resistance in 38 (29.2%) isolates and resistance to ciprofloxacin was high and present in 71 (53.4%) of all isolates.

The ribotype of the four culture-positive, toxin-negative isolates was not determined but all these four isolates had the same antibiotic susceptibility profiles with sensitivity to ceftriaxone, ciprofloxacin, metronidazole and vancomycin.

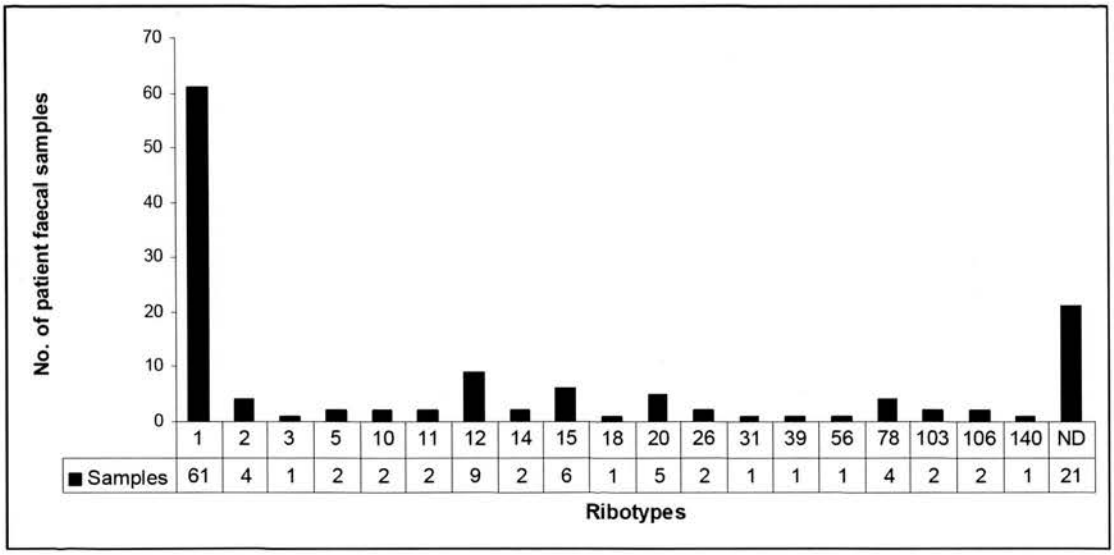


Figure 5.1 The distribution of ribotypes found in the colorectal surgery in-patient faecal isolates.

Table 5.2 (below) Demonstrates the toxigenic culture positive samples, the ribotypes obtained from each sample (ND indicates not determined) and their antibiotic susceptibility profiles for the antibiotics ceftriaxone, ciprofloxacin, metronidazole and vancomycin.

Faecal Sample	ET Toxin Result	Culture	Toxin	Ribotype	Ceftriaxone	Ciprofloxacin	Metronidazole	Vancomycin
XE177653Z	Positive	Positive	Y	001	S	R	S	S
XE177742D	Negative	Positive	Y	001	S	R	S	S
XE177743L	Negative	Positive	Y	010	R	S	S	S
XE177755L	Negative	Positive	Y	001	I	R	S	S
XE177811M	Negative	Positive	Y	005	R	R	S	S
XE177849S	Positive	ReculturePositive	Y	001	R	R	S	S
XE177850F	Positive	Positive	Y	001	I	R	S	S
XE178067V	Negative	Positive	Y	001	R	R	S	S
XE178507W	Positive	Positive	Y	001	R	R	S	S
XE178676J	Positive	Positive	Y	056	S	S	S	S
XE179223C	Positive	Positive	Y	001	R	R	S	S
XE179361R	Negative	Positive	Y	001	R	R	S	S
XE179422G	Positive	Positive	Y	001	R	R	S	S
XE179606V	Negative	Positive	Y	001	R	R	S	S
XE179732Y	Positive	Positive	Y	001	I	R	S	S
XE179876D	Negative	Positive	Y	031	S	S	S	S
XE180136F	Negative	Positive	Y	001	R	R	S	S
XE180748T	Positive	Positive	Y	001	R	R	S	S
XE180825P	Positive	Positive	Y	020	S	S	S	I
XE180846H	Negative	Positive	Y	001	R	R	S	S
XE180847Y	Positive	Positive	Y	012	R	S	S	S
XE180849P	Negative	Positive	Y	001	R	R	S	S
XE181223H	Negative	Positive	Y	002	I	S	S	S
XE181622W	Negative	Positive	Y	001	R	S	S	S
XE181695X	Positive	Positive	Y	003	R	R	S	I
XE182037K	Positive	Positive	Y	001	R	R	S	S
XE182162D	Negative	Positive	Y	103	I	R	S	I
XE182655A	Positive	Positive	Y	001	R	R	S	S
XE182661N	NT	Positive	Y	001	S	S	S	S
XE182742Q	Negative	Positive	Y	103	R	R	S	I
XE182987K	NT	Positive	Y	001	R	R	S	S
XE183762M	Positive	Positive	Y	001	R	R	S	S
XE184516N	Negative	Positive	Y	001	R	R	S	S
XE184544S	Positive	Positive	Y	001	S	S	S	S
XE184641K	Negative	Positive	Y	001	I	S	S	S
XE184866H	Negative	Positive	Y	001	S	S	S	S
XE185160V	Positive	Positive	Y	001	S	S	S	S
XE185161B	Negative	Positive	Y	010	S	S	S	S
XE185380C	NT	Positive	Y	002	I	R	S	S
XE185710G	Negative	Positive	Y	ND	R	R	S	S
XE185975T	Negative	Positive	Y	ND	R	R	S	S
XE185976K	Positive	Positive	Y	106	R	R	S	I
XE186192H	Positive	Positive	Y	012	R	R	S	S
XE186772C	Positive	Positive	Y	001	R	R	S	S
XE186788J	NT	Positive	Y	001	I	R	S	S

XE186789V	NT	Positive	Y	001	I	R	S	S
XE186834V	NT	Positive	Y	001	R	R	S	S
XE186841F	NT	Positive	Y	012	R	R	S	S
XE186861Y	Positive	Positive	Y	001	R	R	S	S
XE187798H	Negative	Positive	Y	140	R	R	S	S
XE188335Q	Positive	Positive	Y	001	R	R	S	S
XE188411J	Negative	Positive	Y	011	I	S	S	S
XE188617T	Positive	Positive	Y	ND	I	S	S	S
XE188856L	Negative	Positive	Y	001	I	R	S	S
XE189008H	Positive	Positive	Y	001	I	R	S	S
XE189029W	Positive	Positive	Y	001	I	R	S	S
XE189103R	NT	Positive	Y	001	I	R	S	S
XE189144V	Positive	Positive	Y	001	I	R	S	S
XE189187L	Positive	Positive	Y	001	I	R	S	S
XE189510Z	Negative	Positive	Y	012	S	S	S	S
XE189511N	Positive	Positive	Y	001	I	R	S	S
XE189554Q	Positive	Positive	Y	001	I	R	S	S
XE189826X	NT	Positive	Y	001	I	R	S	S
XE190634E	Negative	Positive	Y	015	S	R	S	S
XE190730B	Negative	Positive	Y	001	I	R	S	S
XE190799J	Positive	Positive	Y	001	I	R	S	I
XE190881Q	Positive	Positive	Y	001	I	R	S	S
XE191148M	Negative	Positive	Y	001	I	R	S	S
XE191293Q	Positive	Positive	Y	014	S	S	S	S
XE191318M	Negative	Positive	Y	005	S	S	S	S
XE191936Y	Negative	Positive	Y	012	S	S	S	S
XE191938P	Negative	Positive	Y	001	I	R	S	S
XE192186A	Positive	Positive	Y	012	S	S	S	S
XE192199F	Negative	Positive	Y	001	S	S	S	S
XE192238N	Negative	Positive	Y	001	I	R	S	S
XE192340A	Positive	Positive	Y	020	S	S	S	S
XE192637E	Positive	Positive	Y	020	S	S	S	S
XE192966H	Negative	Positive	Y	ND	S	S	S	S
XE193019R	Negative	Positive	Y	015	S	S	S	S
XE193875S	Negative	Positive	Y	ND	S	S	S	S
XE193883R	Negative	Positive	Y	012	R	S	S	S
XE193940B	Negative	Positive	Y	012	S	S	S	S
XE194128W	NT	Positive	Y	001	I	R	S	S
XE194192F	Negative	Positive	Y	001	S	R	S	S
XE194197J	Negative	Positive	Y	001	I	S	S	S
XE194448D	Positive	Positive	Y	026	S	S	S	S
XE194469B	Negative	Positive	N	ND	S	S	S	S
XE194712G	Negative	Positive	Y	001	I	R	S	S
XE194903V	Positive	Positive	Y	001	I	R	S	S
XE195079R	Negative	Positive	Y	ND	S	S	S	S
XE195471L	Negative	Positive	N	ND	S	S	S	S
XE195479W	Negative	Positive	Y	ND	S	R	S	S
XE195591T	Negative	Positive	Y	001	S	R	S	S
XE195946P	Negative	Positive	Y	039	R	S	S	S
XE196079F	Negative	Positive	Y	ND	S	S	S	S
XE196086H	Positive	Positive	Y	ND	S	S	S	S
XE196195D	Negative	Positive	Y	018	S	S	S	S
XE196446N	Negative	Positive	Y	011	S	S	S	S
XE198488Q	Negative	Positive	N	ND	S	S	S	S

XE198544N	Negative	Positive	N	ND	S	S	S	S
XE199902L	NT	Positive	Y	012	S	R	S	S
XE200230V	Negative	Positive	Y	ND	S	R	S	S
XE200522Y	Positive	Positive	Y	026	S	S	S	S
XE200527C	Positive	Positive	Y	078	S	S	S	S
XE201185G	Positive	Positive	Y	078	S	S	S	S
XE201406Y	Negative	Positive	Y	ND	S	S	S	S
XE201511V	NT	Positive	Y	078	I	R	S	S
XE201759T	NT	Positive	Y	ND	I	S	S	S
XE201814H	Negative	Positive	Y	020	S	S	S	S
XE201875S	Negative	Positive	Y	020	S	S	S	S
XE203884V	Negative	Positive	Y	ND	S	S	S	S
XE204308N	Positive	Positive	Y	ND	S	R	S	S
XE205076T	Negative	Positive	Y	ND	S	S	S	S
XE205175Y	Negative	Positive	Y	106	I	R	S	S
XE205293E	Negative	Positive	Y	078	S	S	S	S
XE205321F	Positive	Positive	Y	001	I	R	S	S
XE205452Z	Positive	Positive	Y	001	R	R	S	S
XE205784H	Negative	Positive	Y	ND	S	S	S	S
XE206131Z	Positive	Positive	Y	015	S	S	S	S
XE206250G	Negative	Positive	Y	002	S	S	S	S
XE206616K	Negative	Positive	Y	ND	S	S	S	S
XE206800L	Negative	Positive	Y	001	I	R	S	S
XE206887R	Negative	Positive	Y	002	S	S	S	S
XE207256S	Negative	Positive	Y	001	I	R	S	S
XE207368B	Negative	Positive	Y	015	S	S	S	S
XE207368B	Negative	Positive	Y	015	S	S	S	S
XE207421T	Positive	Positive	Y	001	I	R	S	S
XE207996N	Negative	Positive	Y	001	I	R	S	S
XE209383J	Negative	Positive	Y	014	S	S	S	S
XE209853X	Negative	Positive	Y	015	S	S	S	S

Toxinotyping was performed on 21 of the ribotype 001 isolates from the faecal samples towards the beginning of the study i.e. the first 21 isolates of ribotypes 001 listed in table 5.2. Nineteen were of toxinotype 0 with one variant toxinotype IV. A toxinotype could not be accurately determined from the band patterns obtained from the isolate of sample XE179223C.

5.2.5 *Clostridium difficile* re-infection, relapse and recurrence rates in the colorectal surgery in-patient population.

There was a male preponderance in the entire culture positive patient cohort with 59 male to 43 female adult in-patients. The median age of the entire culture positive cohort was 78 years (range 26 to 100 years).

Six toxigenic, culture-positive patients had two faecal samples sent for testing within 28 days of each other. Both these samples for each patient yielded different toxin producing ribotype *C. difficile* strains (table 5.3). The samples were sent to the enteric laboratory over a range of 1 to 21 days. Ribotypes 001 and 012 were found in three of the six patients with the others distributed as shown in table 5.3.

Patient	Ribotype identified in the first sample	Time period between sent faecal samples (days)	Ribotype identified in the second sample
1	001	21	012
2	001	1	012
3	010	1	012
4	001	8	012
5	012	21	078
6	106	4	001

Table 5.3 The different ribotypes identified in six patients within a 28 day period.

Five patients demonstrated some evidence of re-infection, relapsing or recurrent disease during their in-patient admission in colorectal surgery. Over varying time periods multiple toxigenic *C. difficile* strains were obtained from these five patients isolates. Each patient had two to five samples sent over a period of 53 to 149 days (table 5.4). All these patients had diarrhoea.

P	1st I	Days 1st – 2nd	2nd I	Days 2nd – 3rd	3rd I	Days 3rd – 4th	4th I	Days 4th – 5th	5th I
7	ND	25	015	20	012	8	015	-	-
8	020	21	103	7	103	32	001	-	-
9	011	149	078	-	-	-	-	-	-
10	002	58	002	6	ND	-	-	-	-
11	001	1	001	26	001	2	001	29	001

Table 5.4 The ribotypes identified from different isolates sent from each patient over varying periods of time, with the isolates separated by the time period between each set of intervening samples. (P = patient, I = Isolate obtained from each faecal sample).

Patients 9 and 10 were discharged home between their first and second samples and their second isolates were obtained from their following admission. The presence of different isolates between their first and second samples suggests re-infection with new *C. difficile* strains. Patient 11 due to the persistent identification of ribotype 001 during their single prolonged admission suggests re-lapsing disease. Patient 7 had two ribotypes identified during their admission with ribotype 015 found in the middle and end of periods when they had symptomatic disease, it is possible therefore the disease causing strain was 015 causing relapsing disease and they were carriers of ribotype 012. Again multiple strains were identified in patient 8 and it is likely they were re-infected with different strains during their admission.

5.3 Discussion

The *C. difficile* asymptomatic carriage rate of out-patients attending colorectal surgical services was 6.1%. This figure is in accordance with other studies reporting carriage rates in healthy adults of 3% to 15% (Kato *et al.*, 2001; Kelly & Lamont, 1998). High asymptomatic carriage rates of 14% have also been reported in colonised individuals at the time of their hospital admissions (Kyne *et al.* 2000). Within the in-patient colorectal population a potential *C. difficile* carriage rate of 3.9% was identified on exclusion of patients with symptomatic disease, whilst 16.6% of the patient cohort in this study (80 of 483 patients) had or developed symptomatic CDI during their admission without taking into account relapsing or recurrent disease. Similar figures were reported by Loo *et al.*, (2011) on reviewing 4143 adult patients, they identified 4.4% as asymptomatic carriers at admission and found 3% had health-care associated *C. difficile* colonisation.

A more recent study by Rea *et al.* (2012) found carriage rates of *C. difficile* changed from 1.6% in the community to 9.5% in the out-patient setting and increased to 21% in hospital, although they studied carriage rates in an older population aged 67 to 91 years with a mean age of 83 years, the median age of patients in our cohort was 78 years. Kyne *et al.* (2000) have also reported much higher in-patient acquisition carriage rates of 40%. Using a colorectal surgical population Lesperance *et al.* (2011) reviewed 695,010 hospitalisations during which colonic resections were performed and found a secondary rate of in-hospital acquired symptomatic CDI of 1.4%.

In this study two of the six patients in the asymptomatic out-patient carrier population went on to develop symptomatic CDI following elective admission for major colorectal intra-abdominal surgery. Therefore community-acquired asymptomatic *C. difficile* carriage becomes part of the CDI burden once patients are hospitalised. Due to the inevitable exposure to a host of risk factors hospitalised community acquired carriers are at risk of in-patient symptomatic CDI. In the cases mentioned pre-operative antibiotic prophylaxis and intra-abdominal surgery were the most likely risk factors. (During the period of this study a single dose of ceftriaxone and metronidazole were given intravenously in the peri-operative period for major intra-abdominal elective surgery). Lanzas *et al.* (2011) developed a compartmental model

which showed that transmission of CDI among patients within the ward alone could not sustain the numbers of new *C. difficile* colonisations found within in-patients and therefore new admissions of already colonised patients played an important role in sustaining transmission on the wards.

Asymptomatic carriage in neonates is well recognised dating back to the original identification of *C. difficile* by Hall and O'Toole in 1935; however high carriage in neonates (Al-Jumaili *et al.*, 1984) and infants is now recognised as a potential reservoir for toxigenic strains (Rousseau *et al.*, 2012). There is therefore further cross-over in *C. difficile* colonisation between adults and children in the community, with adults at greater risk of symptomatic CDI development than children once hospitalised.

The flux, in all the studies mentioned and including the cohort reviewed in this study, between carrier rates in the community/out-patient to hospital settings shows the importance of the reservoir and burden of *C. difficile* and the potential implications for management and infection control within individual specialties, when colonised patients are admitted and when colonisation as an in-patient occurs.

Six hundred and thirty-two faecal samples from 483 adult colorectal surgical in-patients were reclaimed over the study period from the enteric laboratory for toxigenic culture. The EL found 9.7% of the faecal samples to be toxin positive by commercial EIA testing for toxins A and B. On toxigenic culture 20.4% of the faecal samples were positive i.e. a further 68 faecal samples were identified as toxigenic *C. difficile* positive. Four faecal samples were culture positive but non-toxin producing. Of the 61 EL toxin positive faecal samples only 77% of these faecal samples were toxigenic culture positive on primary culture. EIA toxin detection has been reported to falsely identify CDI in 1 to 2 samples of every ten positive tested (Wilcox *et al.* 2009 and 2012).

EIA therefore was 52% less sensitive then toxigenic culture for detection of toxin-producing *C. difficile* in this sample population, with a potential false positive proportion of 23% of all the EL toxin positive samples identified which could have had implications for individual patients, and a false positive proportion of 2.5% of all

the 571 faecal samples tested on excluding the sub-set of faecal samples not-tested by the EL (due to duplicate requesting, non-diarrhoeal samples and repeat requesting within 28 days of a positive sample). Fifty-eight patients with diarrhoeal samples were not identified as toxin positive by the enteric laboratory at the time the faecal samples were sent, over the period of the study.

Similar to this study, low sensitivity for toxigenic *C. difficile* detection by EIA for toxins A and B has been reported by other studies. Crobach et al. (2009) provided a systematic review of studies comparing reference standards (cell culture cytotoxic assay or toxigenic culture) to EIA for toxins A and / or B, GDH and RT-PCR. It was found that EIA detecting toxins A and / or B compared with toxigenic culture reported sensitivities which ranged from 0.32 to 0.79 and the specificities ranged from 0.84 to 1.00. They also found that all the commonly applied hospital diagnostic available methods of EIA for toxins and GDH and RT-PCR were not suitable as stand-alone tests to diagnose CDI in endemic populations.

In recognition of the low sensitivities of commonly used tests, a two-step diagnostic algorithm for CDI was recommended by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) in 2009. In their algorithm if EIA is used to detect toxins A and B and the result is negative, if there is no clinical suspicion then no CDI can be documented for the patient, if there is a high clinical suspicion then their samples should proceed to toxigenic culture. In the patients with a toxin positive result, EIA for GDH, RT-PCR or cytotoxic assays should then be performed, if these results are also positive then CDI is diagnosed if not no CDI. In late 2009 the local enteric laboratory adopted a modified version of this which was 9 months after the period of the study reported here. Instead of only EIA to toxins A and B, EIA for GDH is also performed for toxin positive patients. In toxin negative patients where high clinical suspicion of CDI persists then EIA for GDH is performed +/- culture.

Nucleic acid amplification tests (NAATs) are more sensitive then EIA detection kits and were developed as more accurate diagnostic tests for CDI and are molecular tests that detect genes encoding toxin A and B and are now commercially available however they are also unable to differentiate between symptomatic CDI and

colonisation and therefore Wilcox (Dec 2012) reported the limitations of a *positive C. difficile* NAAT result, after a patient has a primary episode of CDI if it is to be used as a single test for CDI.

Repeated samples for individual patients were sent to the local enteric laboratory for repeated EIA toxin testing during the study period. Of the 58 patients that were EL EIA toxin negative, 17 patients had had two repeated faecal samples sent within a period of 1 to 22 days that were all EL toxin negative and toxigenic culture positive. Repeat sample testing during the same symptomatic episode for individual patients has been found not to increase the rate of detection in the endemic setting (Crobach *et al.*, 2009; Bouza, 2012) Garimella *et al.*, 2012) also found decreased clinical utility in repeat testing for hospitalised patients although recognised a possible benefit in outbreak settings where the yield of repeat testing was 5%. Clinical recognition and suspicion of CDI is extremely important in both requesting tests for CDI and for interpretation of any test results (Dubberke *et al.*, 2011).

Screening of other hospital acquired infections (HAI) is now common such as for MRSA (Lee et al., 2010) and active surveillance of MRSA and vancomycin resistant enteroccocus (VRE) has resulted in reduced in-patient rates of these hospital-acquired infections (Muto et al., 2011). In regards to active surveillance for *C. difficile*, Bartsch *et al.*, (2012) evaluated the potential economic value of screening hospital admissions for *C. difficile* and screening was found to be cost-effective in a population with a colonisation rate of $\geq$ 10.3%. At present however any cost-effective and patient benefit to screening is limited by toxigenic *C. difficile* testing as described. In order to identify and isolate carriers of *C. difficile* prior to elective admission a sensitive, specific, rapid cost-effective test needs to be readily available. EIA GDH and NAATs have been suggested due to a greater ability to detect *C. difficile* in a low CDI prevalence population such as the community. Curry *et al.* (2011) suggested peri-rectal swab surveillance with selective broth amplification and RT-PCR as a further possible method. The definite benefit of screening for *C. difficile* in the UK has yet to be fully investigated.

On analysis of the 133 isolates identified as culture positive, 4 were found to be non-toxin producing isolates and a ribotype for these isolates was not determined but all

were found to have the same antibiotic profiles with sensitivity to all four of the antibiotics tested. A further 3 of the 133 isolates could not be grown from their stores. Of the remaining 126 isolates all were found to be toxigenic *C. difficile* strains. The commonest ribotype, within this colorectal surgical population, was 001 found in 46.9% of all samples and this is consistent with other local studies, where ribotype 001 had emerged as the most common ribotype locally over the last decade. McCoubrey *et al.* (2003) used a S-layer typing method to type isolates collected from South-East Scotland from 1999 to 2000 and found 73% of isolates to be S-type 5236 which is equivalent to ribotype 001. An increased incidence in ribotype 001 from 1.5% to 12.2% over a grouped period from 1979 to 2004 was found by Taori *et al.* (2010) who also assessed isolates from South-East Scotland, and Mutlu *et al.* (2007) examined 149 isolates from different hospitals in the Edinburgh area in 2005 and reported an incidence of 75.8%. Conversely in Scotland as a whole, over the period isolates were collected for this study, ribotype 106 was the predominant strain (Health Protection Scotland 2009). Despite Mutlu *et al.* (2007) identifying ribotype 106 in 8.1% of their isolates, ribotype 106 was found in only 1.5% of isolates in this study. A specific cohort of patients was assessed in this study and therefore may not represent local changes across Lothian.

The incidence of CDI in Scotland is now closely monitored since mandatory data reporting was introduced in September 2006 for those aged over 65 years and in 2009 mandatory data reporting was increased to include those aged 15 years and over. The first surveillance report produced by Health Protection Scotland identified ribotypes 106 (64%) and 001 (18.5%) as the two predominant ribotypes between 2006 and 2007 and from 2007 to 2008 the prevalence of ribotype 001 rose to 24.5%. In 2009 ribotypes 106 and 001 remained the most common ribotypes with the emergence of 027 and 078 also noted. Ribotype 078 was found in 3% of our isolates. Towards the end of 2010 the emergence of 015, 002, 014, 020 and 005 were also noted. All these ribotypes were found in small incidences within the colorectal population studied. More recently in 2012, ribotype 078 has become the prevalent ribotype in Scotland.

Ribotype 012 was the next commonest strain identified in this study, after 001, and was found in 6.9% of isolates. Ribotype 012 was also the third commonest isolate, comprising 5% of the isolates, in the study by Taori *et al.* (2010). Ribotype 012 is

representative of a local epidemiological strain and it is not of significant prevalence in the rest of Scotland.

Overall 19 ribotypes were identified from the colorectal surgical population and this is comparable to Mutlu et *al.*, (2007) who reported 15 different ribotypes within their cohort; however 21 colorectal in-patients isolates ribotypes were not determined by the end of this study. Twenty-one of the 001 ribotype isolates from earlier on in the study were also assessed with toxinotyping with 19 of the isolates of toxinotype 0. This is also similar to Mutlu et *al.*, (2007) who found 96.6% of their isolates to be of toxinotype 0.

All the toxigenic *C. difficile* isolates were also assessed for their sensitivity or resistance to four antibiotics ceftriaxone, ciprofloxacin, metronidazole and vancomycin. Metronidazole remains the mainstay of antibiotic treatment for CDI particularly in patients deemed to have mild to moderate disease. Locally, mild to moderate disease is characterised by an absence of the following severity markers; leucocytosis >15 x 10^9 cells/l, creatinine > 1.5 x patient's baseline, pyrexia >38°C, albumin ≤25g/l, elevated lactate, suspected or confirmed PMC, ICU admission or immunosuppression. In the presence of one or more severity markers vancomycin is advocated and with PMC and or fulminant CDI metronidazole and vancomycin are given. Only a few *C. difficile* strains have been reported to have metronidazole resistance and increases in metronidazole MICs have also been observed (Brazier *et al.*, 2001, Pelaez *et al*, 2002, Baines *et al.*, 2008).

All the 130 *C. difficile* isolates tested in this study were sensitive to metronidazole and this is comparable to other studies (Aspevall *et al.* 2006; Taori *et al.*, 2010). An MIC of 4μg/ml was demonstrated in 6 isolates (4.6%) denoting these isolates with intermediate resistance to vancomycin and was found in isolates belonging to ribotypes 103, 001, 020 and 106. The remaining 127 isolates were all sensitive to vancomycin. Other studies have also demonstrated reduced susceptibility to vancomycin with MICs of 4ug/ml (Liao *et al.*, 2012) and at a local level Drummond *et al.* (2003) identified 2.6% of their isolates had MICs of 4μg/ml to vancomycin and Mutlu *et al.*, (2007) found 21.6% of their isolates to have intermediate resistance to vancomycin.

Overall ceftriaxone resistance was found in 35 of 130 isolates (26.9%) and intermediate ceftriaxone resistance in 38 (29.2%) isolates. Resistance to ciprofloxacin was high and present in 71 (53.4%) of all isolates. Studies have reported the unnecessary use of flouroquinolones particularly for treatment of urinary tract infections and asymptomatic bacteriuria (Werner 2011), which will also be driving increased resistance to these antibiotics. Many studies use moxifloxacin as the fluoroquinolone to test *C. difficile* antibiotic susceptibility, however in this study ciprofloxacin was used. This was because ciprofloxacin is widely prescribed within the UK and moxifloxacin has never been used locally. Therefore this study provides further information on ciprofloxacin fluoroquinolone resistance in *C. difficile*, which is rarely reported on.

Thirty isolates demonstrated resistance to both ceftriaxone and ciprofloxacin, whilst 31 isolates were resistant to ciprofloxacin with intermediate resistance to ceftriaxone. Studies have reported a higher resistance in dominant ribotypes. In this study ribotype 001 was the dominant strain with 93% of isolates demonstrating resistance to ciprofloxacin and 95% demonstrating resistance or intermediate resistance to ceftriaxone. Mutlu *et al.,* (2007) also found higher resistance to ceftriaxone and moxifloxacin among their dominant ribotypes 001 and 106, and Taori *et al.,* (2010) found ribotype 001 displayed the maximum resistance with 50% of isolates resistant to erythromycin, ceftriaxone and moxifloxacin. The next common ribotype 012, demonstrated resistance to ceftriaxone in 4 isolates (44.4%) and ciprofloxacin resistance in 3 isolates (33.3%). Spigaglia *et al.* (2011) also demonstrated multidrug resistance in their cohort of ribotype 012 isolates.

Six toxigenic culture positive patients yielded two different ribotypes from two faecal sample isolates sent for testing within 28 days of each other. Two of a combination of the ribotypes 001, 012 010, 078 and 106 were found in the 6 patients. Multiple strains within the same patient at the same time have been previously reported (van den Berg *et al.*, 2005). As only one isolate was obtained from each faecal sample it is likely that if multiple *C. difficile* colonies within the faecal samples were evaluated the yield for multiple strains in individual patients would have been much higher. Although treatment of disease clinically is the same regardless of the strain, in this locale where

ribotype 027 is not prevalent, epidemiologically the strain causing disease and why the other strain remains dormant is of interest. Furthermore does the patient's risk of recurrence due to re-infection increase in the presence of multiple strains?

Recurrence is the term used to cover both re-infection from a different *C. difficile* strain and recurrence from the same strain, as clinically it is virtually impossible to tell the difference without epidemiological typing data. Recurrence rates for CDI can vary from 18% to 25%, and these patients often develop symptoms within days to weeks of stopping treatment for their first CDI episode (Bauer et al., 2011; Louie et al., 2011). In this study 5% (5 of 99 patients from the toxigenic culture positive cohort) demonstrated some evidence of re-infection, relapsing or recurrent disease during their in-patient admission in colorectal surgery. Multiple toxigenic *C. difficile* strains were obtained from these five patients and each patient had 2 to 5 samples sent over a period of 53 to 149 days, and a combination of the following ribotypes were found 015, 012, 020, 103, 001, 011, 078, 002 and 001, in two of the isolates the ribotypes were not determined. Two patients were discharged home between their first and second isolates and differing ribotypes suggested re-infection with a new *C. difficile* strain. One patient had persistent identification of ribotype 001 during their lengthy admission suggesting re-lapsing disease. Two patients were found to have varying ribotypes within their isolates over a significant period of time. Other studies have also shown re-infection rates with new strains, some in up to 50% of recurrences (Poxton 2013; van den Berg *et al.*, 2005). Although no metronidazole or vancomycin resistance was demonstrated in the isolates from the relapsing/recurrent disease patient cohort the recognition of these patients is important when considering treatment options. Fidaxomicin a newer antibiotic for *C. difficile* treatment has been shown to be more effective than vancomycin in treating recurrent disease (Poxton 2010) and is now being advocated for use in regimes after vancomycin in patients with multiple recurrences (Johnson *et al.*, 2013). Another treatment option for recurrent CDI is faecal microbiota transplant which has also been shown to be of benefit in recurrent CDI (Gough *et al.*, 2011). Currently tapering doses of vancomycin are used to treat relapsing CDI however these newer treatment options could be considered.

6. *Clostridium difficile* and the environment in colorectal surgery.

6.1 Introduction

The environment is an important reservoir for *Clostridium difficile* including toxigenic strains; therefore the environment plays an important role in the transmission of CDI. *C. difficile* is a spore-forming organism and it is these spores that exist in the environment and facilitate the transmission of disease.

C. difficile is spread by the faecal-oral route and therefore *C. difficile* spores and vegetative cells must be ingested into the gastro-intestinal (GI) tract for disease to occur. *C. difficile* spores colonise primarily the colon of the GI tract and under the correct conditions following exposure to certain risk factors, the spores are able to germinate into vegetative cells. The vegetative cells produce toxins and other virulence factors which result in the cytotoxic and inflammatory response of the colonic mucosa resulting in symptomatic disease, with diarrhoea the primary presenting symptom in most cases. The vegetative cells also produce more spores and these shed into the environment whereby the spores can then be ingested by another patient thus allowing a perpetual cycle of transmission from the host to the environment and the environment to the host. *C. difficile* spores and vegetative cells shed in huge quantities into the environment from infected symptomatic patients (Mulligan et al., 1980; Wilcox et al., 2003). Asymptomatic carriers also shed spores and vegetative cells but not to the degree demonstrated in patients with symptomatic diarrhoea (Kim *et al.*, 1981; Samore *et al.*, 1999). Healthy asymptomatic carriers treated with antibiotics for non-CDI related pathology also shed large volumes of *C. difficile* which is reversed at the completion of the antibiotic course (Chachaty *et al.*, 1993).

The human reservoir of *C. difficile* includes both asymptomatic carriers and those with symptomatic disease. Asymptomatic carriers as a reservoir for *C. difficile* have long been established particularly in the neonatal and infant populations (Hall & O'Toole 1935; Holst *et al.*, 1981; Matsuki *et al.*, 2005). The asymptomatic carriage rate in healthy adults of both toxigenic and non-toxigenic strains ranges from 2% to

14% (Kyne *et al.*, 2006) and of toxigenic strains from 3% to 7% (Ryan *et al.*, 2010). Epidemic strains are found in asymptomatic carriers and therefore they may contribute to the spread of nosocomial CDI (Riggs et al., 2007), as previously discussed in Chapter 5. Asymptomatic carriage has also been documented in hospital patients with no previous antibiotic exposure history or antibiotic associated diarrhoea (Varki & Aquino 1982).

The cohorts of patients with the greatest *C. difficile* reservoir are those who have symptomatic disease. Direct contact in a shared room with a patient with symptomatic CDI is a significant risk factor for the transmission of nosocomial disease to patients who were previously not carriers of *C. difficile* (Chang *et al.*, 2000). Patients in rooms neighbouring those containing patients with symptomatic CDI are also at greater risk of CDI development (Chang *et al.*, 2000). Other factors that also increase the risk of CDI transmission among ward patients include antibiotic usage (Rotimi *et al.*, 2002; Fenton *et al.*, 2008) and duration of hospitalisation (Rudensky et al., 1993).

In hospitals the surfaces, food, healthcare workers and visitors the patients come into contact with are all part of the environmental reservoir for disease transmission. *C. difficile* acquisition by health-care workers is uncommon, despite the high level of contact health-care workers often have with symptomatic CDI patients. Fewer than fifteen cases of CDI in health-care professionals deemed to be work-related have been reported (Dorn, 2009). Some studies have also found no carriage of *C. difficile* in heath-care workers who were in direct contact with CDI patients (Carmeli *et al.*, 1998). Transfer of *C. difficile* between symptomatic patients to family contacts and the transmission of *C. difficile* between healthy family members has also been observed (Sutphen *et al.*, 1983; Kato *et al.*, 2001). Therefore patients and their family members/visitors are at potential risk of acquiring *C. difficile* from each other.

The potential spread of nosocomial disease via contaminated hospital surfaces has been demonstrated. Following the isolation of hospital associated organisms from many surfaces found within the hospital environment it is accepted that the surface environment within hospitals and long-term care facilities, are a potential source of hospital-acquired infections (HAI) disease transmission. *C. difficile* has been cultured from many surfaces within the hospital setting including floor, mops, bed-linen, sluice

rooms, computers, telephones, tables, doorknobs, air vents, medication carts and uniforms (Kim *et al.*, 1981; Fawley & Wilcox 2001; Dumford *at al.*, 2009; Perry *et al.*, 2001).

Vegetative *C. difficile* cells are at higher concentrations prior to the start of antibiotic treatment than during and at the end of treatment (Jump et al 2007). However, unlike vegetative *C. difficile* cells which are eliminated by treatment antibiotics, environmental shedding of *C. difficile* in particular spores often persists after symptom resolution and can persist after treatment (McFarland *et al.*, 1998 and Sethi *et al.*, 2010). *C. difficile* vegetative cells can survive on moist surfaces for 3 to 6 hours and occasionally up to 12 hours in aerobic conditions (Weber et al., 2010). *C. difficile* spores, however, are hardier entities and are able to survive on surfaces for several months; *C. difficile* spores have been isolated from floors for up to five months following artificial inoculation (Kim *et al.*, 1981). They are also fairly resistant to external environmental changes such as temperature (Rodriguez-Palacios *et al.*, 2010) and a patient's internal defence mechanisms such as the acidic low pH of their gastric contents.

C. difficile spores are resistant to a variety of disinfectant agents especially in the presence of organic matter whilst vegetative cells are more susceptible to the effect of sporicides and survive on surfaces for much shorter periods of time than spores (Jump *et al.*, 2007; Wheeldon *et al.*, 2008). Therefore cleaning agents targeted at *C. difficile* must be both bactericidal and sporicidal.

The aims of this study were:

1. To determine the degree of environmental *C. difficile* surface contamination on the colorectal surgical wards with residing colorectal surgical in-patients namely wards 22, 23, 24 and 27 at the Western General Hospital, Edinburgh.
2. To assess changes in *C. difficile* environmental surface contamination on the colorectal surgical wards over a period of one year.
3. To determine changes in the level of *C. difficile* environmental surface contamination and the number of *C. difficile* positive colorectal in-patients following implementation of a new cleaning strategy.

4. To assess the epidemiology of *C. difficile* strains found in the surface environment of colorectal surgical wards and in the colorectal surgical in-patients admitted to these wards during the period of study.

6.2 Materials and Methods

Wards 23, 24 and 27 all had a similar layout with each ward containing four main bays and five side-rooms. Each main bay contained five patient bed spaces each with a separate toilet room with a sink and a separate shower room contained within each main bay. The side-rooms contained one patient bed space with its own en-suite facilities. Ward 22 contained two main bay areas with four patient bed spaces per bay and four side-rooms. The main bays and not the side rooms were used for environmental sampling, as the side rooms were often used for isolation of symptomatic *C. difficile* toxin positive patients. Side-room cleaning is generally more stringent once the side-room is vacated compared with the main patient bay areas and it is the main bays where CDI transmission between patients is most likely to occur (Chang *et al.*, 2000). Most patients will have been in a main bay area prior to transfer to a side-room when symptomatic CDI as an in-patient developed. Therefore in order to determine accurate background levels of *C. difficile* contamination particularly in areas that were generally considered more low risk, sampling of side-rooms containing a symptomatic *C. difficile* toxin positive patient would have resulted in falsely higher results.

Following a pilot study, sites were identified for sampling, due to the poor yield obtained from fabric during the pilot study with contact plates; it was elected not to sample bed linen, the segregating bed-space curtains or the window curtains. Within each main bay; the bed frame, bedside locker and bedside table per patient bed space was sampled and the main bay toilet room door handle and main bay toilet room sink were also sampled. The communal main bay sink handles in the corner of each bay mainly for use by staff but also patients and visitors were also sampled. Other communal areas and equipment sampled included the floors in the main corridors of each ward and the communal blood pressure cuffs of all the sphygmomanometers on each ward. On one occasion a patient did not want their bed frame sampled and therefore the patient's bed hand control was sampled instead.

The original study period for environmental sampling of the colorectal surgical wards was initially from January 2008 to January 2009 inclusive. This meant that the in-patient *C. difficile* positive colorectal surgical population study (Chapter 5) results would also include the same time period and could be used for comparison.

Following completion of the original study, it was elected to continue the environmental sampling portion of the study until the end of March 2009, to determine if any changes to environmental contamination, within the colorectal surgical wards, following introduction of a new cleaning strategy could be maintained. Therefore any comparisons pre and post introduction of the new cleaning protocol had to include primarily the same duration before and after the new protocol was introduced. Similarly as colorectal in-patient surgical faecal samples were only collected to the end of January 2009 comparisons involving the in-patient cohort could only involve the original study period.

The colorectal surgical in-patient cohort used for comparison in this study was identified from all the patients found to be *C. difficile* toxigenic culture positive from January 2008 to January 2009 inclusive, from the study described in Chapter 5. To allow direct comparison in this study only those patients that were in-patients on wards 22, 23, 24 and 27 for a minimum of one night were included. Colorectal surgical boarders i.e. those patients under the care of the colorectal surgical team admitted to different specialty wards for a variety of reasons including lack of available surgical beds, requirements for isolation, transfer to another clinical team with continued surgical input etc. were not included as these wards' surface environment were not sampled. Toxigenic culture positive patients that were likely to have transited through the colorectal surgical wards without overnight admission were included in the comparative *C. difficile* strain analysis. Materials and Methods are otherwise as described in Chapter 2.

6.3 Results

6.3.1 *Clostridium difficile* contamination in the colorectal surgical wards.

Over the study period 72 (4%) of 1800 environmental contact plates were culture positive for *C. difficile* and of these 70 environmental contact plates contained toxigenic strains of *C. difficile*.

Ward 24 had the greatest proportion of environmental positive contact plates with 40% of all positive plates, followed by ward 23 with 31.4%, ward 27 with 21.4% and ward 22 with 7.1% (Figure 6.1). Using the same patient cohort from Chapter 5, 99 colorectal in-patients were toxigenic culture positive. On excluding those patients that were colorectal surgical in-patient boarders and toxigenic culture positive patients out with the period of the original environmental study, 62 patients were in-patients on wards 22, 23, 24 and 27 from 1st January 2008 to 31st January 2009. Similar to the environmental positive samples, ward 24 had the greatest number of *C. difficile* toxigenic culture positive patients. However, ward 22 with the next highest number of toxigenic culture positive patients had the lowest number of positive environmental samples (Figure 6.1).

C. difficile colonies were obtained from individual patient bed frames, lockers, tables and a patient bed hand held control; the communal floors; ward bay patient shared areas including toilet room door handles and the toilet room sink handles; a ward bay general communal used clean sink and communal sphygmomanometers (Figure 6.2). Bed frames were the commonest sites of *C. difficile* contamination with 44.2% of all culture positive environmental samples obtained from the bed frames. Patients' bedside lockers, patients' bedside tables and the communal floors were the next commonest sites with 12.8% of culture positive samples each. A further 7.1% of all the positive samples were obtained from the communal patient sphygmomanometer cuffs.

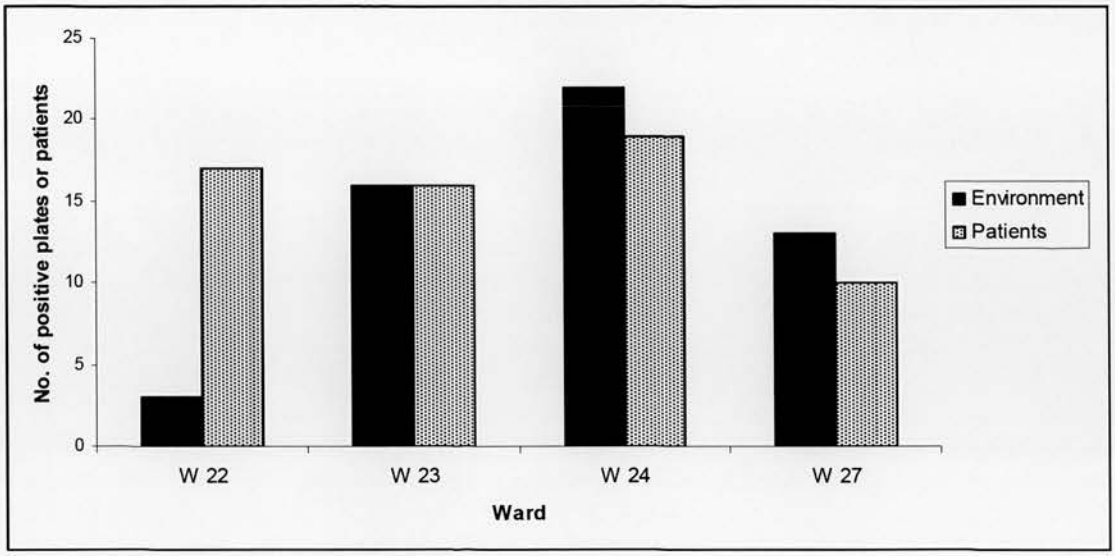


Figure 6.1 The number of *C. difficile* culture positive environmental contact plates obtained from each ward and the number of *C. difficile* positive in-patients residing in each ward over the study period (W = ward).

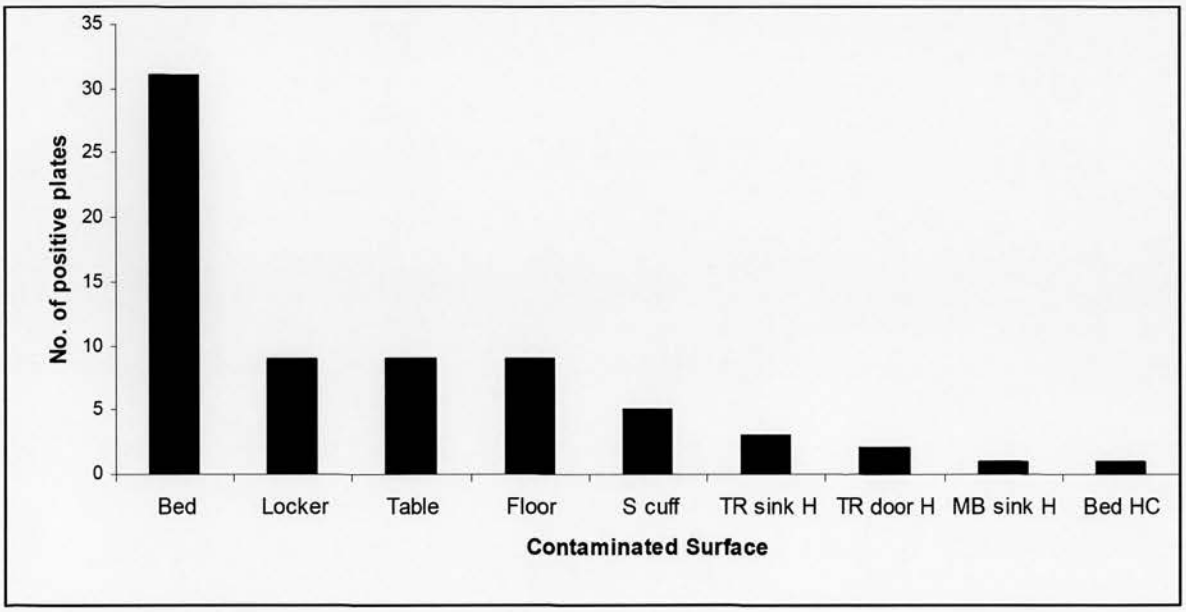


Figure 6.2 The number of culture positive environmental contact plates obtained from each surface over the study period (S = sphygmomanometer cuff, TR = toilet room, H = handle, MB = main bay, HC = hand control).

6.3.2. Characterisation of *Clostridium difficile* isolates obtained from the colorectal surgical ward surface environment.

Of the 72 culture-positive environmental plates only one *C. difficile* colony was identified on each of 70 plates. On two plates two *C. difficile* colonies were present, resulting in 74 environmental *C. difficile* culture positive isolates. Seventy-one isolates were toxin producing. From the contact plates which had two colonies; on one plate one of the colonies was a non-toxin producing *C. difficile* strain and on the other plate both colonies were toxin producing however one of the colonies could not be cultured from its Robertson's cooked meat broth store during the period of MIC antibiotic susceptibility testing and the period when cultures were being sent to the Health Protection Agency, Colindale for typing. This isolate has therefore not been included in this part of the study.

Of the three non-toxin producing isolates the ribotype could not be determined and these three isolates had the same antibiotic susceptibility profiles with sensitivity to ceftriaxone, ciprofloxacin, metronidazole and vancomycin.

Over the environmental study period of the toxin-producing *C. difficile* isolates ribotype 001 was the most common ribotype found from the colorectal ward environmental isolates with 34 of the 70 isolates (48.6%) identified as ribotype 001. Ribotypes 020 (7.1%) and 026 (7.1%) were the next commonest found in 5 of the 70 isolates each, followed by ribotype 002 (4.3%) (Table 6.1 and figure 6.3). Further ribotypes 012, 015, 039, 081, 103, 106 and 176 were identified in the remaining isolates. A ribotype was not determined in 10 of the 70 isolates (14.3%).

All ribotype isolates were sensitive to both metronidazole and vancomycin.
The 34 isolates identified as ribotype 001 demonstrated variable resistance to ceftriaxone and ciprofloxacin. Twenty-six of the 34 ribotype 001 isolates showed intermediate resistance to ceftriaxone and all of these 26 isolates were resistant to ciprofloxacin. One ribotype 001 isolate was resistant to both ceftriaxone and ciprofloxacin and the remaining seven ribotype 001 isolates were sensitive to ceftriaxone with 6 of these resistant to ciprofloxacin and one sensitive to ciprofloxacin. Resistance to ciprofloxacin was almost ubiquitous in the ribotype 001

environmental isolates with resistance to ciprofloxacin demonstrated in 97% (33 out of 34) of these isolates.

In the environmental isolates with the next most common ribotypes 020 and 026, all the ribotype 020 isolates were sensitive to ceftriaxone and ciprofloxacin, whilst intermediate resistance to ceftriaxone was found in two of the five ribotype 026 isolates and ciprofloxacin resistance in one of the 026 isolates.

Overall intermediate ceftriaxone resistance was found in 39 of 70 isolates (55.7%) and ceftriaxone resistance in 2 (2.9%) isolates and resistance to ciprofloxacin was high and present in 46 (65.7%) of all isolates.

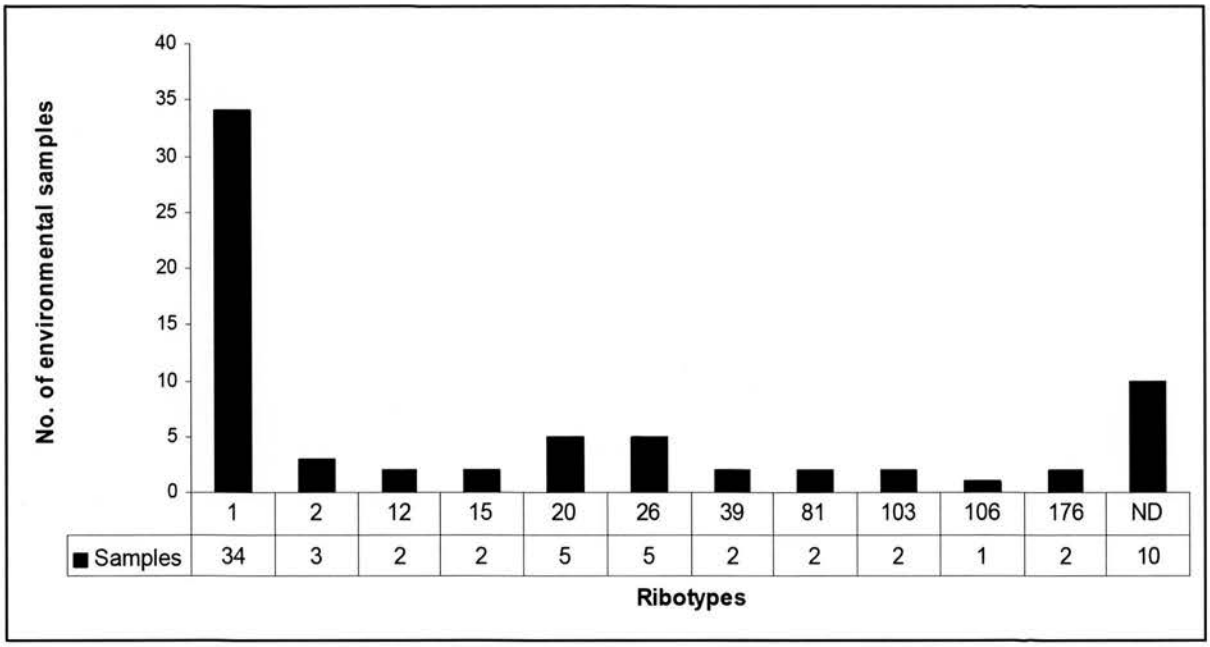


Figure 6.3 The distribution of ribotypes found in the colorectal surgical wards environment isolates.

E Number	Date	ward	surface	ribotype	toxin	Ceftriaxone	Ciprofloxacin	Metronidazole	Vancomycin
1	Jan-08	23	TS	12	Y	S	R	S	S
2	Jan-08	23	F	1	Y	I	R	S	S
4	Jan-08	24	B	1	Y	I	R	S	S
5	Jan-08	24	B	1	Y	I	R	S	S
6	Jan-08	23	B	12	Y	S	R	S	S
8	Jan-08	24	B	26	Y	S	S	S	S
9	Jan-08	23	B	1	Y	I	R	S	S
10	Jan-08	22	B	ND	Y	I	S	S	S
11	Jan-08	24	B	103	Y	I	S	S	S
14	Mar-08	24	bp	1	Y	S	R	S	S
15	Mar-08	24	T	1	Y	S	R	S	S
16	Mar-08	24	B	1	Y	I	R	S	S
17	Mar-08	24	T	1	Y	I	R	S	S
18	Mar-08	23	B	1	Y	I	R	S	S
20	Mar-08	23	F	1	Y	I	R	S	S
21	Mar-08	27	TH	1	Y	S	R	S	S
22	Mar-08	27	B	15	Y	S	R	S	S
24	Mar-08	27	F	1	Y	I	R	S	S
25	Mar-08	22	L	1	Y	I	R	S	S
29	May-08	24	F	1	Y	I	R	S	S
30	May-08	24	B	1	Y	I	R	S	S
31	May-08	24	bp	2	Y	I	S	S	S
32	May-08	24	bp	2	Y	S	S	S	S
33	May-08	24	bp	103	Y	S	R	S	S
34	May-08	23	bp	1	Y	S	R	S	S
35	May-08	24	B	1	Y	I	R	S	S
36	May-08	24	L	26	Y	I	S	S	S
37	May-08	23	L	1	Y	I	R	S	S
38	May-08	23	T	20	Y	S	S	S	S
41	May-08	23	L	20	Y	S	S	S	S
42	May-08	27	TH	ND	Y	S	R	S	S
43	May-08	27	B	20	Y	S	S	S	S
44	May-08	27	L	ND	Y	S	S	S	S
45	May-08	27	B	1	Y	I	R	S	S
47	May-08	22	F	20	Y	S	S	S	S
48	Jul-08	23	B	1	Y	I	R	S	S
49	Jul-08	23	B	20	Y	S	S	S	S
53	Aug-08	24	F	1	Y	S	S	S	S
54	Aug-08	24	T	81	Y	I	S	S	S
55	Aug-08	24	T	1	Y	I	R	S	S
56	Aug-08	24	B	1	Y	I	R	S	S
57	Aug-08	24	L	81	Y	S	S	S	S
58	Oct-08	27	B	1	Y	I	R	S	S
60	Oct-08	27	B	ND	Y	I	S	S	S
63	Oct-08	27	TS	ND	Y	S	S	S	S
65	Oct-08	27	B	15	Y	S	S	S	S
66	Oct-08	27	B	1	Y	I	R	S	S
67	Nov-08	24	B	1	Y	I	R	S	S
69	Nov-08	24	T	106	Y	I	R	S	S
72	Nov-08	23	B	176	Y	I	R	S	S
74	Nov-08	23	T	26	Y	S	S	S	S
78	Nov-08	23	T	176	Y	I	R	S	S
79	Nov-08	23	TS	ND	Y	S	S	S	S

82	Jan-09	27	B	39	Y	I	S	S	S
90	Jan-09	27	B	1	Y	I	R	S	S
93	Jan-09	27	F	ND	Y	S	S	S	S
96	Feb-09	24	B	1	Y	S	R	S	S
98	Feb-09	24	B	2	Y	R	R	S	S
99	Feb-09	24	L	1	Y	I	R	S	S
100	Feb-09	24	B	1	Y	I	R	S	S
101	Feb-09	24	B	ND	Y	S	S	S	S
103	Feb-09	24	L	39	Y	I	R	S	S
104	Mar-09	23	F	26	Y	I	R	S	S
105	Mar-09	23	BS	ND	Y	I	R	S	S
106	Mar-09	23	B	ND	Y	S	R	S	S
107	Mar-09	23	HC	1	Y	I	R	S	S
109	Mar-09	23	B	26	Y	S	S	S	S
110	Mar-09	23	L	1	Y	I	R	S	S
111	Mar-09	22	T	1	Y	R	R	S	S
112	Mar-09	22	F	1	Y	S	R	S	S

Table 5.1 Demonstrates the environmental toxigenic culture positive isolates, the ward and surface from which they were obtained, the ribotypes obtained from each isolate (ND indicates not determined) and their antibiotic susceptibility profiles for the antibiotics ceftriaxone, ciprofloxacin, metronidazole and vancomycin. (B = bed, bp = sphygmomanometer blood pressure cuff, BS = ward main bay sink, F = floor, HC = patient bed hand control, L = patient bedside locker, T = patient bedside table, TS = main bay patients' toilet room sink handle and TH = main bay patients' toilet room door handle).

6.3.3 The impact of introduction of a chlorine-based cleaning agent in the distribution of *Clostridium difficile* in colorectal surgery.

In early June 2008 a new cleaning protocol, as previously described in section 2.7.4 of the Materials and Methods, was introduced with the major change involving cleaning of vacated bed spaces with Acti-chlor, a chlorine based cleaning agent, rather than quaternary ammonium compounds and /or bleach detergents. During the month of June 2008 there was a transition period as staff, both ward and domestic staff, got used to using Acti-chlor. Therefore no environmental samples were collected during June 2008 to accommodate the change and environmental sampling re-commenced in July once changes were fully implemented and established.

Environmental sampling was performed in January, March, May, July, August, October and November 2008 and January, February and March 2009. The distribution of environmental toxigenic *C. difficile* plates over the entire environmental study period is shown in Figure 6.4. The maximal number of positive plates and hence isolates were obtained in May 2008. During the period prior to implementation of the new cleaning protocol 19.4% of the environmental contact plates used were positive for toxigenic *C. difficile* and for the same duration following the new cleaning protocol only 2.3% of the environmental contact plates used were positive. There was a 48.6% reduction in toxigenic *C. difficile* environmental contamination following the new cleaning protocol compared with the same time period prior to the change. However, only a 25.7% reduction was demonstrated between the extended study periods in 2009 compared with the similar time period prior to the change in cleaning protocols.

Over the original environmental study period January 2008 to January 2009 inclusive, there was a statistically significant reduction in environmental contamination after the new cleaning procedures were introduced, $p = 0.0189$, compared with the old cleaning protocol. Similarly there was a statistically significant reduction in toxigenic culture positive in-patients on the colorectal wards, $p = 0.0026$, for the period following the new cleaning protocol compared with the period prior to its introduction (Figure 6.5).

On reviewing the number of toxigenic *C. difficile* positive environmental plates and the number of toxigenic culture positive patients per ward per month, clustering of the positive plates and patients is demonstrated (Figure 6.6), with an increased number in both positive plates and patients prior to the introduction of the new cleaning protocol on each ward.

Over the entire environmental study period, on assessing the number of toxigenic *C. difficile* environmental contact positive plates per ward per month (Figure 6.7), it was noted that prior to the introduction of the new cleaning protocol, that all the wards with colorectal surgical patients generally had toxigenic *C. difficile* contamination each month. Following the new cleaning protocol *C. difficile* contamination was only detected on a single ward or a maximum of two wards per month, July 2008 to March 2009 inclusive, rather than on all the wards. Similarly, on reviewing the number of

toxigenic culture positive in-patients on the colorectal wards, there was a distribution of positive patients on all of the wards during the period of the old cleaning protocol. However, once the new cleaning protocol was established, from July 2008 to January 2009 there was an overall decreased distribution of patients across the wards with patients generally found on the wards where environmental *C. difficile* contamination had been identified (Figure 6.6).

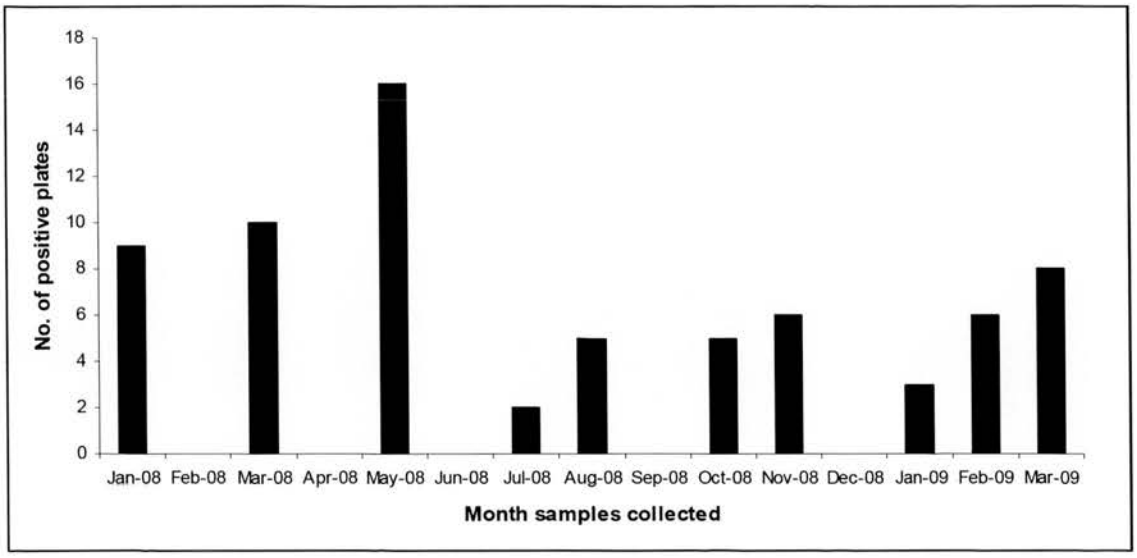


Figure 6.4 The distribution of toxigenic positive *C. difficile* environmental plates over the study period.

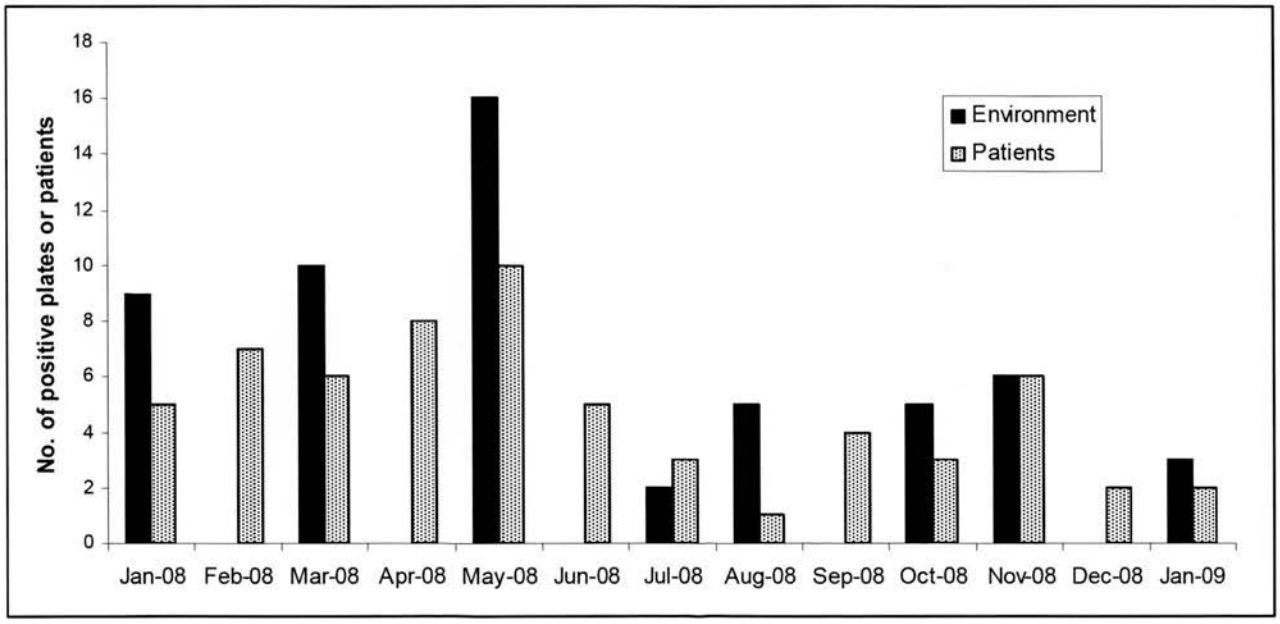


Figure 6.5 The distribution of the number of *C. difficile* culture positive environmental contact plates obtained and the number of *C. difficile* positive in-patients per month over the study period.

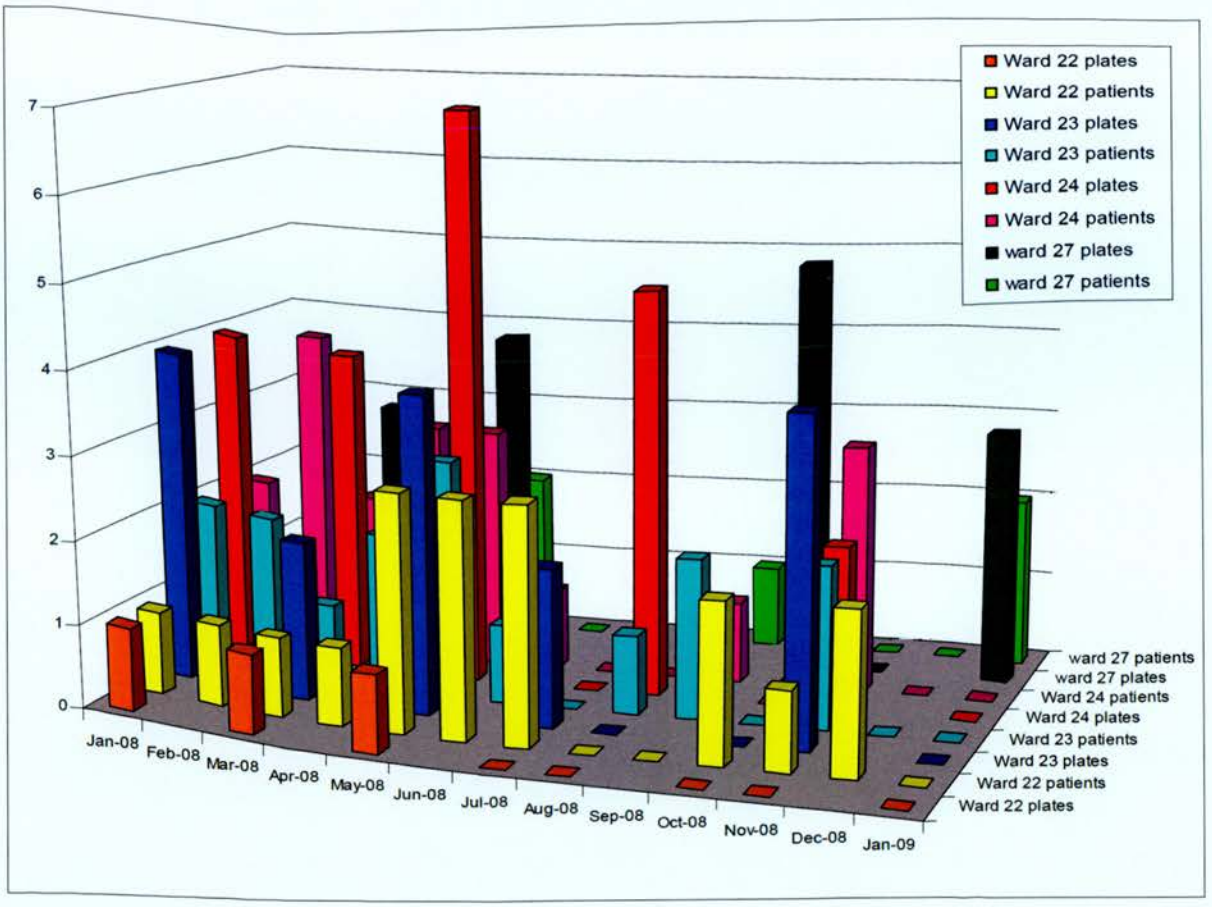


Figure 6.6 The number of *C. difficile* culture positive environmental contact plates obtained and the number of *C. difficile* positive in-patients on each ward per month of the study period.

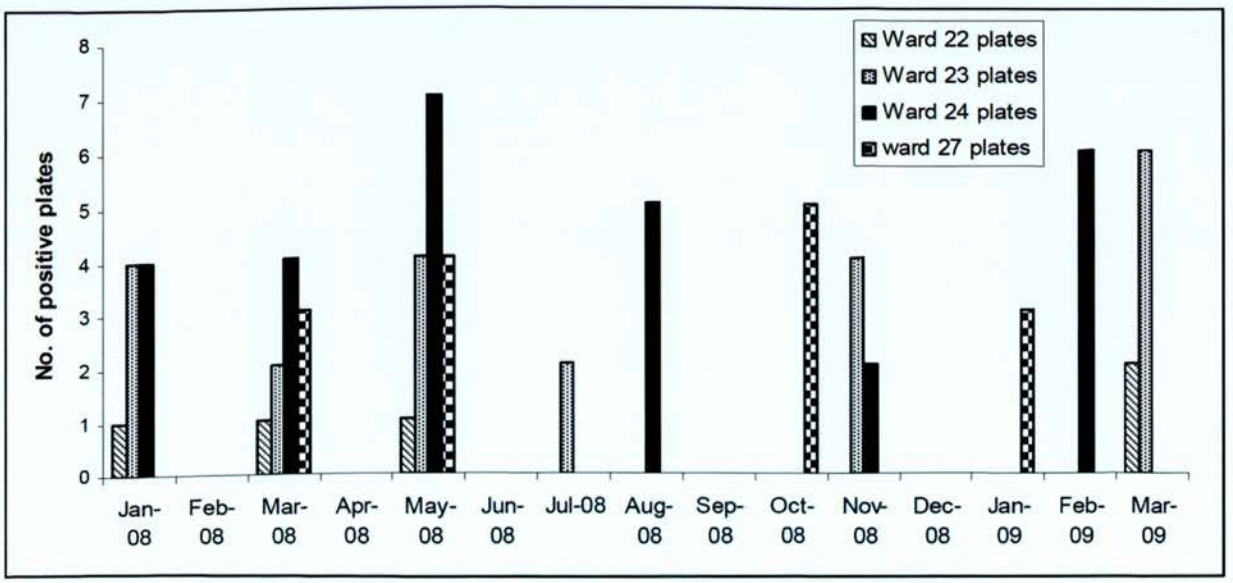


Figure 6.7 The distribution and number of toxigenic *C. difficile* positive environmental contact plates obtained from each ward for each month of the study.

6.3.4 Distribution of *Clostridium difficile* ribotypes pre and post introduction of the new cleaning protocol.

Eleven different toxigenic *C. difficile* ribotypes were identified in the colorectal surgical ward environment over the entire environmental study period. The distribution of the number of different ribotypes identified in the colorectal surgical ward environment pre and post the new cleaning protocol did not change with eight different ribotypes identified prior to the new cleaning protocol and nine different ribotypes identified after its introduction. However, the proportion of ribotype 001 isolates did decrease by 57.6% over the original study period (January 2008 to January 2009), pre and post the new cleaning protocol. A recurrent slow rise in the prevalence of 001 in the environment was noted however from January to March 2009 inclusive (Figure 6.8).

In relation to the environmental isolates, the proportion of in-patients with ribotype 001 also decreased by 65.7% over the original study period, pre and post establishment of the new cleaning protocol. When taking into account all the toxigenic culture positive isolates from patients who passed through the colorectal wards during the study or were treated by the same colorectal clinical teams including those boarded to other non-colorectal wards and those patients with recurrent disease seventeen different toxigenic *C. difficile* ribotypes were identified in the patient population from January 2008 to January 2009. Again the number of different ribotypes identified in the patient cohort pre and post the new cleaning protocol did not change with 14 different ribotypes identified prior to the new cleaning protocol and 13 different ribotypes identified after its introduction (Figure 6.9).

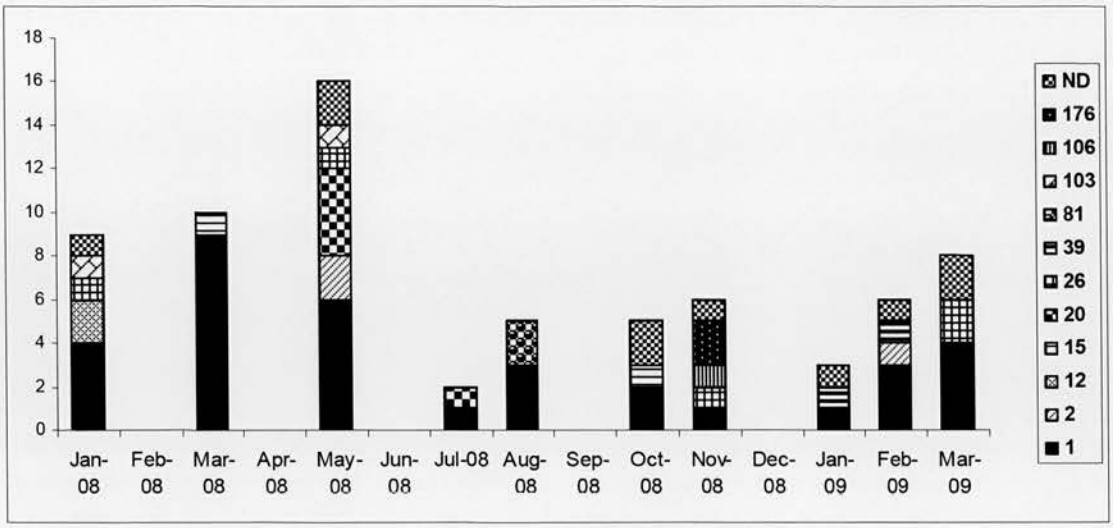


Figure 6.8 Distribution of the different environmental toxigenic *C. difficile* ribotype isolates identified over the entire environmental study period.

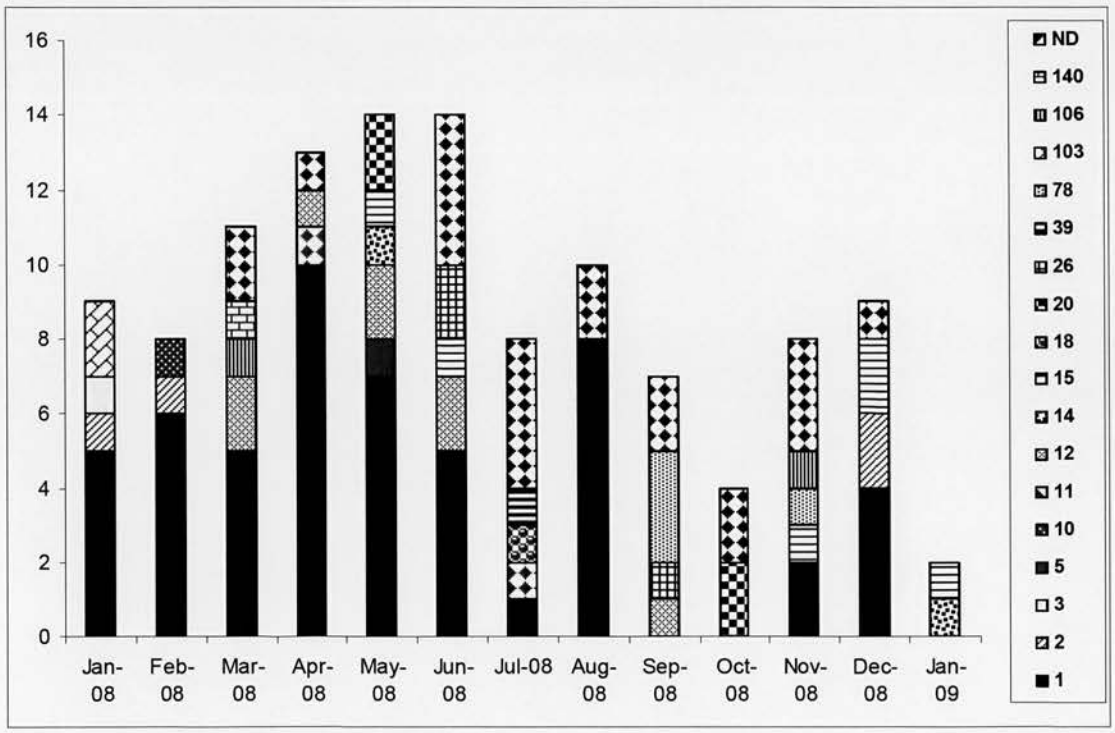


Figure 6.9 Distribution of the different toxigenic culture positive *C. difficile* patient isolates identified over the original environmental study period.

6.4 Discussion

The bed frames were the greatest site of *C. difficile* contamination with 44.2% of all positive environmental samples obtained from the bed frames. Two other studies also found the bed frames to be the most common environmental site from which *C. difficile* was recovered (Verity *et al.*, 2000; Guerrero *et al.*, 2012). The patients' bed side lockers and tables also had reasonable levels of *C. difficile* contamination with a combined proportion of 25.6% of the total number of *C. difficile* positive environmental samples. These are surfaces upon which the patient's food and belongings were kept and therefore were a high risk for transmission of CDI as the spores were more likely to be ingested off these surfaces. All the *C. difficile* contamination relating to the patient bed spaces were in main bay areas and not from isolation rooms in which *C. difficile* positive patients had been isolated. Therefore the patients occupying these bed spaces and their visitors were at risk of CDI acquisition be it asymptomatic acquisition or symptomatic disease. In addition, potential forgotten reservoirs of *C. difficile* in the environment, in particular the bed frames were highlighted.

C. difficile contamination obtained from the wards' main communal floors was present in 12.8% of all positive environmental samples. The importance of the presence of *C. difficile* and other bacteria on floors has been debated and two studies have shown that the disinfection of floors played a negligible role in the transmission of disease and had no impact on rates of infection (Fawley & Bond 2001; Danforth *et al.*, 1987). Similarly Voss *et al.*, 2003 also suggested that routine disinfection of floors was not necessary unless required in certain situations such as contamination of floors with organic matter. Arguments in favour of floor disinfection include implementation of a single cleaning strategy including floors ensures standardisation and uniformity of cleaning and training procedures for staff (Rutala & Weber, 2001), and Exner et al., (2004) using an experimental model found that cleaning of floors with a mop using detergents resulted in the spread of bacteria from contaminated areas to clean areas and therefore intimated that disinfection including floors should be part of a complete approach to hospital hygiene. With *C. difficile* spores able to exist on floors for up to 5months (Kim et al., 1981) their presence adds to the contamination burden present within a hospital environment and provides an

indication of background hygiene levels within a hospital environment. Objects can be dropped on the floor and picked up further allowing for potential transmission of spores or spores may be aerosolised from the floor into the air.

Approximately 7% of all the positive environmental samples were obtained from the communal sphygmomanometer arm cuffs. This equated to five blood pressure cuffs from two wards identified in March and May of 2008 prior to the new cleaning protocol. Following the new cleaning protocol *C. difficile* was not isolated from the cuffs. Manian et al. (1996) found unexpected contamination of blood pressure cuffs with *C. difficile* at a rate of 10% which was similar to that for bedside commodes. In their study, an observational survey had revealed that healthcare workers not infrequently forgot to remove their gloves before touching clean surfaces. Despite their study occurring over a decade ago, it is interesting that similar findings occur.
Two other commonly touched sites by patients, staff and visitors are the main bay sink handles and the patient bed hand control, *C. difficile* was only identified from these on one occasion each, although the hand control had only been assessed once during the entire study as an alternative to the bed frame. Dumford *et al.*, (2009) found of the surfaces they sampled; 21% of portable medical equipment such as medication carts and portable computers were contaminated with *C. difficile* and that 10% of surfaces in the nursing work area and 31% of surfaces in medical staff work areas were also contaminated.

C. difficile contamination on the hands of healthcare workers on wards containing patients with symptomatic CDI has been previously recognised (Fekety *et al.*, 1981; Samore *et al.*, 1996), and correlation between transmission of CDI between patients and contamination of the hands of healthcare workers has been described (McFarland *et al.*, 1989). Taffinder *et al.* (1997) sampled infants in a neonatal intensive care unit over a period of 12 months and showed 57% of infants were colonised at first sampling dropping to 10% latterly as infection control procedures primarily hand hygiene measures were introduced and therefore cross-infection was most likely to be occurring by handlers. Corresponding to these, Samore *et al.* (1996) found that contamination of the hands of health-care workers directly related to levels of environmental contamination. Therefore the hands of health-care workers are

generally considered the primary means of *C. difficile* spore transmission in health-care settings (Bobulsky *et al.*, 2008).

Sethi *et al.*, 2010 determined that skin contamination and environmental shedding of *C. difficile* remained common at the time of resolution of diarrhoea, although the proportions were significantly reduced compared to the proportions before treatment. They also found more than half their patient cohort tested 1 to 4 weeks after treatment for CDI had asymptomatic stool carriage and concurrent skin and environmental contamination. Acquisition of *C. difficile* from investigators' hands in their study continued to occur 50% of the time after contact with the patient's chest, abdomen, arm and hand. In a further study by Guerrero et al., (2012) on assessing a cohort of patients with symptomatic CDI, they found risk of hand contamination after contact with commonly examined skin sites and commonly touched environmental surfaces was identical. The abdomen and groin were the two examined sites which caused the greatest hand contamination and the bed rail, the environmental site which caused the greatest hand contamination. Therefore equipment that is not disposable such as communal sphygmomanometer cuffs, portable ultrasound machines and stethoscopes etc. are still at risk of contamination even after the patient is asymptomatic and is no longer in isolation. Alleyne *et al.*, 2009 isolated *C. difficile* from 4.9% of stethoscopes.

C. difficile spores were isolated from the main bay patient toilet room door handles and the toilet room sink handles. These are areas that potentially *C. difficile* would expect to be isolated from as *C. difficile* environmental culture recovery rates have been shown to be greater from toilet and sluice room floors (Fawley et al., 2001). In this study, whilst these areas accounted for 5.7% of all positive environmental samples combined they were only the sixth most common surface identified with *C. difficile* contamination. This may be a reflection of good hand hygiene in the patient population generally. The higher levels of contamination identified on the patient's bed space surfaces, as described earlier, are therefore not necessarily from the patient themselves, as colonised asymptomatic carriers, but remain present following vacation of a symptomatic CDI patient from that bed-space previously or transmission by hands of staff.

Persistence of *C. difficile* environmental contamination may relate to recent evidence of aerial dissemination of spores, and therefore improved ward ventilation may also help to reduce CDI transmission. Fawley *et al.*, (2001) cultured *C. difficile* from air vents. Roberts *et al.*, (2008) isolated *C. difficile* from the air using a portable cyclone sampler, from a six bedded elderly care bay over a period of two days. They found that large numbers of particles were found in the air at almost all time points and therefore *C. difficile* aerosolisation events occurred throughout the day. Best *et al.*, (2010) found *C. difficile* was more frequently isolated from the air during periods of activity such as ward rounds and visiting hours. Recently Best *et al.*, (2012) assessed the affect of aerosolisation following toilet flushing and found *C. difficile* was recoverable up to a height of 25cm above the toilet seat whilst surface contamination occurred 90 minutes after flushing, therefore aerosolisation following flushing resulted in seeding of the surrounding environment and the simple act of flushing with the toilet lid closed could reduce environmental and aerosolised *C. difficile.*

Ward 24 had the greatest proportion of environmental positive contact plates and therefore not surprisingly had the greatest number of *C. difficile* toxigenic positive patients. However, ward 22 with the next highest number of toxigenic culture positive patients had the lowest number of positive environmental samples. Whilst this does in part reflect good cleaning procedures, the differences may also be a result of admission cohort and procedures. Ward 22 often admitted the acute admissions generally for a maximum 24 hour period and then primarily admitted both colorectal and urology day case patients. The colorectal surgical admissions to Ward 22 would then be delegated to Ward 23, 24 or 27 for longer stay or discharged home. Therefore patients with potential symptomatic CDI admitted as emergencies or day cases will have had their faecal specimen sent from and recorded as ward 22 but were likely rapidly transferred to another colorectal ward, therefore decreasing the time period over which environmental shedding and contamination could occur.

During the period preceding implementation of the new cleaning protocol 19.4% of the environmental contact plates used were positive for toxigenic *C. difficile.* Dumford *et al.* (2009) similarly found 16% of their samples from rooms of patients not in isolation for CDI were culture positive (their samples were collected approximately one year prior to the commencement of this study). For the same

duration of time following introduction of the new cleaning protocol only 2.3% of the environmental contact plates were toxigenic *C. difficile* positive. This figure is consistent with Faires *et al.*, (2012) who found 2.4% of surfaces they tested were contaminated with *C. difficile* in a study performed approximately one year following the completion of this study. Our figures therefore are likely to be representative of decreased environmental contamination changes happening in other centres of the western world over the last few years during endemic rather than outbreak settings, as awareness increased and improved infection control was introduced.

Over the original environmental study period there was a significant reduction in environmental contamination ($p = 0.0189$) and the incidence of toxigenic culture positive in-patients ($p = 0.0026$) on the colorectal surgical wards following the introduction of the new cleaning protocol. The old cleaning protocol had used a combination of quaternary ammonium compounds and / or bleach (NaOCl) for disinfection and the new cleaning protocol used Actichlor plus (NaDCC) for disinfection at 1000ppm of chlorine either with a detergent pre-clean or in combination with a detergent. NaDCC base agents and bleach are both sporicidal and bactericidal.

Quaternary ammonium compounds have been found to be significantly less effective than other agents as a disinfectant (Dharan *et al.*, 1999, Hacek *et al.*, 2010) and may account for the increased levels of contamination prior to the new cleaning protocol. Sodium hypochlorite (bleach) has been widely used in hospitals however its effectiveness as a disinfectant is dependant on the concentration and the duration of contact it is left in contact with the surface for. Bleach at a concentration of 5000ppm causes a reduction in *C. difficile* spores of 6 $\log_{10}$ in 10 minutes; however, the use of bleach at these concentrations requires protective personal equipment, concentrations of bleach of 3000ppm have to be left for 15 minutes and 1000ppm for up to 25 minutes for a similar reduction in *C. difficile* spores (Perez *et al.* 2005). As bleach is quick-drying, it can be difficult to ensure an even concentration across a surface and therefore decreased cleaning and disinfection may occur particularly if sprayed onto a surface using lower concentrations (Omidbakhsh, 2010). During the period of study using the old cleaning agents it was uncertain at what concentration the agents were being prepared for use and for how long they were left to act on surfaces. Bleach

however when used at concentrations of 5000ppm has been found to be effective in reducing the prevalence density of *C. difficile* patients (Hacek *et al.*, 2010).

Sodium dichloroisocyanurate based agents, such as Actichlor, have been found to be better disinfectants than bleach (Bloomfield & Uso, 1985). Similar to this environmental study Wilcox *et al.*, (2003) found environmental contamination on a hospital ward and incidence rates of CDI decreased with the use of a NaDCC based agent. Wheeldon et al., (2008) found the efficacy of NaDCC was reduced in the presence of organic matter and contact of 9 minutes at a concentration of 1000ppm, as used on the wards in this study, was required to eliminate spores to below the detection limit. Therefore in the presence of organic contamination pre-cleaning with detergent followed by wiping the surface with the NaDCC based agent improves the action of the disinfectant. Conversely Vohra et al., (2011) did not find decreased efficacy of Actichlor, in the presence of organic matter.

There was a 48.6% reduction in toxigenic *C. difficile* environmental contamination following the new cleaning protocol compared with the same time period prior to the change. However, only a 25.7% reduction in environmental contamination was demonstrated on reviewing January to March 2009 compared with the similar time period using the old cleaning protocol. This could reflect an increase in admitted CDI positive patients as the CDI patient population study was not continued beyond January 2009. Locally, previously symptomatic CDI patients are taken out of isolation once diarrhoea has stopped for 48 hours, however as previously demonstrated by Sethi *et al.*, (2010) continued environmental shedding and skin contamination can persist at significant levels for 1 to 4 weeks and the changes in environmental contamination seen at the beginning of 2009 may reflect this. Even with increased incidence, ideally contamination rates should not increase and may therefore also reflect decreased awareness in using the new agents following the initial push after their introduction, such as not leaving the agent on the surface prior to wiping for the recommended period of time and not targeting potential *C. difficile* reservoir surface areas.

Although a slow increase in *C. difficile* environmental contamination was noted from January to March 2009, following the initial sustained decrease from July 2008 to

January 2009, it was noted that, following the new cleaning protocol, *C. difficile* contamination was only detected on a single ward or a maximum of two wards per month. Similarly there was an overall decreased distribution of patients across the wards, with patients generally found on the wards where environmental *C. difficile* contamination had been identified. Prior to the new cleaning protocol all the wards generally had toxigenic *C. difficile* contamination and toxigenic culture positive in-patients. Therefore environmental contamination and the presence of CDI positive patients do not generally exist without the other once good cleaning, isolation and protective measures are established. Kim *et al.*, (1981) found in areas where *C. difficile* patients had diarrhoea 9.3% of environmental cultures were positive and in areas where there were no known CDI carriers, only 2.6% of cultures were positive. However as previously stated in this study, environmental contamination was not identified in areas where CDI positive patients were absent once the new cleaning protocol was introduced.

Over the environmental study period ribotype 001 was the most common *C. difficile* strain identified from the colorectal ward environmental isolates with 48.6% of all isolates identified as ribotype 001. This is identical to the proportion of toxigenic culture positive faecal isolates from the colorectal surgical in-patients where 46.9% of isolates were identified as ribotype 001. Therefore the environmental epidemiology remains consistent with local epidemiology studies where ribotype 001 has been the prevalent ribotype in Southeast Scotland as described in Chapter 5. Interestingly ribotypes 020 (7.1%) and 026 (7.1%) were the next commonest ribotypes identified from environmental isolates as opposed to ribotype 012 which was the next most common ribotype from the patient faecal isolates.

All environmental isolates were sensitive to both metronidazole and vancomycin, where as in the patient faecal isolates 4.6% had denoted intermediate resistance to vancomycin. Of interest 76.5% of ribotype 001 environmental isolates showed intermediate resistance to ceftriaxone and only one ribotype 001 isolate (2.9%) was resistant to ceftriaxone, however in the patient faecal isolates 36% of ribotype 001 isolates were resistant to ceftriaxone. Similar to the patient faecal isolates ciprofloxacin resistance in the ribotype 001 environmental isolates was high and present in 97%. Overall intermediate ceftriaxone resistance was found in 55.7% and

ceftriaxone resistance in only 2.9% of environmental isolates (compared with 29.2 % and 26.9% respectively in the patient faecal isolates) and resistance to ciprofloxacin in the environmental isolates was high as seen with the patient faecal isolates.

When taking into account the resistance to the tested antibiotics in the environmental isolates versus the patient faecal isolates as a whole the overall antibiotic resistances were similar however definite ceftriaxone resistance was higher in the overall patient faecal isolates in particular the ribotype 001 isolates compared with the environmental group similarly intermediate vancomycin resistance was only demonstrated in the patient isolates. Therefore this poses the question - does increased antibiotic resistance in *C. difficile* develop in patients once they have been infected by a less resistant *C. difficile* strain, due to *C. difficile* exposure to sub-MIC concentrations of antibiotics or do *C. difficile* vegetative cells and spores become less resistant to antibiotics in the environment? This is of particular importance in dominant ribotypes where studies have shown increased antibiotic resistance in dominant strains (Mutlu *et al.*, 2007; Taori *et al.*, 2010).

Other features of ribotype 001 in addition to its multi-drug resistance, may have allowed it to emerge locally as the dominant ribotype and persist. Like other epidemic strains such as 106 and 027 it produces alcohol resistant spores in large quantities and therefore the previous wide-spread use of alcohol based hand gels in the hospital environment provided false reassurance of "clean" hands with decreased washing of hands with soap and water. Higher concentrations of decontaminants and disinfectants including Actichlor have been required to destroy ribotype 001 *C. difficile* vegetative cells (Vohra *et al.*, 2011), again likely pertaining to its resistance mechanisms. Ribotype 001 also has the ability to cause severe disease (Arvand et al., 2009 and Sundram et al., 2009) and although the ribotypes causing disease in the cohort of CDI patients, described in Chapter 3, with pseudomembranous colitis and requiring surgical intervention for CDI were not known, it is likely ribotype 001 was responsible for CDI in a proportion of these patients given that it has emerged as the dominant ribotype locally.

Eleven different toxigenic *C. difficile* ribotypes were identified in the colorectal surgical ward environment compared with seventeen different ribotypes in the patient

faecal isolates. Further adding supporting evidence to Lanzas *et al.*, (2011) experimental model, that transmission of CDI among patients does not occur within the ward alone and new patient admissions already colonised with *C. difficile* play an important role in sustaining the number of patients with CDI on wards.

The proportion of the dominant ribotype 001 isolates in this study decreased markedly after introduction of the new cleaning protocol, by 57.6% in the environment and by 65.7% in the colorectal inpatients. The distribution of the number of different ribotypes identified in the colorectal surgical ward environmental and patient isolates did not change pre and post the new cleaning protocol and neither did any single ribotype become more prevalent as ribotype 001 decreased. In this endemic setting, without notable CDI outbreaks in this population during the study period, improved infection control results in several different strains being able to subsist and remain responsible for CDI in the absence of a dominant strain.

Other cleaning agents and methods have also been studied for use in the environmental disinfection of *C. difficile.* A hydrogen peroxide dry mist system has been found to be effective in the de-contamination of *C. difficile* with reduction rates of 91% to 94% and has been found to be effective against ribotypes 106, 001 and 027 (Barbut *et al.*, 2007; Shapey *et al.*, 2008; Passaretti *et al.*, 2012). Sexton *et al.*, (2011) found portable steam vapour systems reduced bacterial levels by greater 90%, however *C difficile* could not be evaluated as only one colony was found pre cleaning. Smith *et al.*, (2011) found re-usable ultra micro-fibre cloths were efficacious at removing surface organisms associated with HAI including CDI. A UV-C device demonstrated an 80% spore reduction of contaminated surfaces however cycles took up to 45 minutes to achieve eradication of spores, and could not be used in rooms with current in-patients (Nerandzic *et al.*, 2010). A hand-held far-UV radiation device using a spectrum with a higher photon energy than UV-C, showed a significant reduction in *C. difficile* spores when used for only 5 seconds, however it was found to be less effective in the presence of organic material. The biocidal effect of copper as a contact surface that can be applied to a clinical setting also continues to be investigated in laboratory studies (Gant *et al.*, 2007; O'Gorman *et al.*, 2012). The future of *C. difficile* decontamination therefore is still to be determined in the interim this study has shown that good cleaning procedures with a NaDCC based agent can

significantly reduce *C. difficile* environmental contamination and the incidence of CDI positive patients.

7. Conclusions

Clostridium difficile was identified as an infective agent that originated from outwith a patient with *Clostridium difficile* infection and was not simply an overgrowth of part of the commensal colonic flora in the early 1980s (Poxton, 2013). Prior to this in the late 1970s *Clostridium difficile* had been identified as the causative agent of CDI and pseudomembranous colitis (Larson *et al.*, 1977; Bartlett et al., 1978). Since then the incidence and recognition of CDI has increased particularly in the western world over the past four decades and *C. difficile* has become the principal cause of nosocomial diarrhoea in adults and an important cause of antibiotic-associated diarrhoea with CDI associated morbidity and mortality also increasing.

Lothian University Hospitals Division provides for a population of approximately 620 000 people in the Edinburgh area of South-East Scotland, and is a tertiary referral centre for peripheral district general hospitals and in some specialties for all of Scotland. Using locally collected data from 2000 to 2007 this was one of the first studies to assess the *C. difficile* related burden handled by the diagnostic enteric hospital microbiology laboratories and provide the first data on potential clinical workload for an area of Scotland over an eight-year period. The study also provided epidemiological data on a region not affected by ribotype 027, a hypervirulent *C. difficile* strain.

A 27-fold increase in the number of faecal samples analysed by the enteric laboratory occurred from 2000 to 2006 and the total number of potential CDI cases increased over the same period, with a decline finally seen in 2007. With one-fifth of all toxin-positive samples, tested by the enteric laboratory, from age groups under 60 years of age this was further evidence that CDI was not just a disease of the elderly. Although Medicine of the Elderly provided the greatest analysis workload for the enteric laboratory, Renal Medicine / Transplant Surgery, Intensive Care, Infectious Disease and Gastrointestinal Medicine all had higher incidences of CDI than Medicine of the Elderly. Similarly the low risk group of Paediatrics was also starting to show a small but notable increase in potential incidence although these figures were reported with caution due to the high carriage rates previously reported in children. With extra associated costs of approximately £4000 per CDI in-patient case the potential excess

costs for CDI in this region rose from £3.5 million pounds to £29 million pounds over the study period.

The General Surgical specialties had a greater CDI incidence then General Medicine. On closer review of the surgical specialties, colorectal surgery had the greatest number of CDI episodes followed by Upper Gastrointestinal Surgery and Urology. Despite the total number of *C. difficile* toxin-positive in-patients across the Lothian area and the number of colorectal surgical toxin-positive in-patients increasing each year from 2000 to 2006, a similar continued increase was not demonstrated in the number of patients diagnosed with more severe forms of CDI, PMC and fulminant CDI, and similarly an increase was not demonstrated in the number of CDI patients referred and treated with surgical intervention.

Most patients referred for surgical intervention to treat fulminant CDI were referred from other specialties and had received broad-spectrum antibiotics associated with CDI development. Of note, 30% of the surgically treated cohort were emergency admissions from home with probable community acquired CDI. In these extreme cases of CDI up to 40% of patients did not present with diarrhoea and up to 50% of patients did not have a *C. difficile* toxin-positive faecal sample prior to surgery. This demonstrated the importance of clinical recognition of the entire spectrum of *C. difficile* related disease.

The post-operative mortality rate for fulminant CDI was 26% in this cohort, with reported mortality figures in other studies varying from 19% to 64% (Neal *et al.*, 2011; Trudel *et al.*, 1995). High mortality figures for fulminant CDI treated surgically have not changed significantly over the last two decades. Most of the patients in this operative CDI cohort were in extremis, as surgical referral for CDI often occurs late, with earlier surgical referral, surgical management of CDI could become a definitive life-saving rather than a desperate procedure. New operative procedures such as a diverting loop ileostomy with colonic lavage and colonic vancomycin treatment may begin to replace the sub-total colectomy and end ileostomy formation and segmental colectomy procedures currently performed.

An asymptomatic carrier rate of 6.1% was identified in the out-patient colorectal surgical population and an asymptomatic carrier acquisition rate of 3.9% was identified in the in-patient colorectal surgical population. Asymptomatic carriers in particular those admitted from the community play an important role in sustaining the transmission of disease within the hospital environment with 42.8% of *C. difficile* strains identified in the in-patients but not in the environment of the colorectal surgical wards.

CDI diagnosis using enzyme immuno-assay for toxin A+B detection was 52% less sensitive then toxigenic culture with a false positive rate of 2.5%. Toxigenic culture identified a further 58 colorectal surgical inpatients with CDI who were not identified with standard enteric laboratory testing at the time of the study. Since this study was performed in recognition of the diminished sensitivity of EIA alone in other studies the local enteric laboratory uses a modified version of recent European Guidelines. However no one test for CDI testing has been found to be sensitive, rapid, inexpensive and easy to use to completely replace EIA for symptomatic CDI testing, and therefore two-step protocols including the detection of glutamate dehydrogenase and EIA or PCR are currently implemented.

Of all the isolates identified from toxigenic culture-positive CDI in-patients; ribotype 001 was the commonest *C. difficile* strain isolated, consistent with other local studies where ribotype 001 has emerged as the dominant strain. Similarly ribotype 001 was the commonest strain identified in the colorectal surgical ward surface environment. Although ribotype 012 was the next commonest in-patient strain, 020 and 026 were found to be the next commonest environmental strains.

A large proportion of the in-patient *C. difficile* isolates in particular ribotype 001 showed resistance to ceftriaxone and ciprofloxacin. Interestingly the ribotype 001 isolates from the surface environment showed decreased resistance to ceftriaxone compared with the in-patient strains. Similarly 4.6% of all in-patient isolates showed intermediate resistance to vancomycin but no vancomycin resistance was demonstrated in the environmental surface isolates. This may represent increased development of *C. difficile* resistance mechanisms once in the host following sub-MIC exposure to various antibiotics. The study also provided important information

on ciprofloxacin resistance as fluoroquinolone resistance is usually tested with moxifloxacin.

Within the colorectal surgical in-patient cohort 5% of patients were identified with two toxigenic *C. difficile* ribotypes within a 28 day period. Although clinically the treatment of disease remains the same regardless of strain particularly in absence of ribotype 027, epidemiologically multiple toxigenic strains in the same patient poses interesting questions including does a patient's risk of recurrence increase in the presence of multiple strains? A further 6% of patients were found to have recurrent disease over substantial periods of time with multiple *C. difficile* ribotypes and also the same ribotype causing disease in each patient with recurrence. With current treatment of recurrent CDI depending on the use of a tapering vancomycin dose regimen and intermediate vancomycin resistance being demonstrated, newer treatment options such as fidaxomicin and faecal microbiota transplant should also be considered.

The patient bed frames were the commonest contaminated environmental surface with *C. difficile*, followed by the patient's bedside lockers and tables. Therefore the risk of a patient ingesting a *C. difficile* spore from the surface environment is high. During the environmental sampling study a statistically significant reduction in environmental *C. difficile* surface contamination was demonstrated, following the introduction of a new cleaning protocol. Similarly a statistically significant reduction in the number of toxigenic culture positive colorectal in-patients was also demonstrated following the new cleaning strategy which involved replacing quaternary ammonium based compound detergents and bleach with a sodium dichloroisocyanurate based agent, such as Actichlor, with a detergent pre-clean prior to its use. Following the introduction of the new cleaning protocol there was also a marked reduction in the proportion of ribotype 001 isolates in both the environmental surface and colorectal surgical in-patient samples. Therefore ribotype 001 did not remain the dominant ribotype.

In the first study assessing CDI from 2000 to 2007 retrospectively, approximately one-tenth of all toxin-positive patients identified, were transferred through a minimum of two specialties whilst remaining positive for *C. difficile* toxins. The maximum

number of inter-specialty transfers (and therefore wards) for an individual CDI patient was six and this does not take into account the number of individual bed moves within the same ward itself. Reduction of CDI in in-patients also results in decreased environmental contamination and vice versa. Therefore acquisition of CDI from the surface environment in hospitals is not to be under-estimated. With a reasonable proportion of identified CDI patients transferred through different specialties and the significant financial burden CDI imposes on healthcare institutions judicious application of infection control measures remains an important factor in preventing CDI transmission.

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Changes in laboratory and clinical workload for *Clostridium difficile* infection from 2003 to 2007 in hospitals in Edinburgh

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Abstract

Clostridium difficile infection (CDI) is a growing concern with regard to increases in incidence and its associated financial burden. A retrospective analysis of patients admitted to Hospitals in Edinburgh from 2003 to 2007 and tested for *C. difficile* toxins was performed. A total of 45 412 faecal samples were tested and 6286 (13.8%) were positive. Overall CDI was identified in 1.7 cases/1000 in-patient occupied bed days (OBD). The incidence of CDI fell from 1.98 cases/1000 OBD in 2006 to 1.48 cases/1000 OBD in 2007. Renal Medicine, including Transplant Surgery, and Intensive Care had the highest incidence, with >6.2 cases/1000 OBD each, followed by Infectious Diseases and Gastrointestinal Medicine, with rates of 5.5 and 4.42 cases/1000 OBD, respectively. Medicine of the Elderly had an incidence of 1.69 cases/1000 OBD. Incidence increased with age, from 0.45 cases/1000 OBD in the 0–20-year-old age group to 2.02 cases/1000 OBD in the 61–80-year-old age group. Twelve percent of all toxin-positive patients were transferred through a minimum of two specialties when they remained positive for *C. difficile* toxins. Estimated costs over the study period for toxin testing were approximately £126 500 and the minimal potential hospitalization costs for patients with CDI was £20 000 000. The overall incidence of patients identified with CDI fell in 2007 compared to 2006. The incidence has increased with age; however, patients in Medicine of the Elderly had a much lower incidence than in several other specialties and therefore risk assessment of CDI should also be targeted within other specialties. Judicious application of infection control measures remains important for preventing CDI.

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Introduction

Clostridium difficile infection (CDI) has increased over the past three decades and is the principal cause of nosocomial diarrhoea in adults and an important cause of antibiotic-associated diarrhoea. CDI covers a spectrum of disease from asymptomatic carriage, mild self-limiting diarrhoea, through to severe diarrhoea, pseudomembranous colitis and toxic megacolon, with the possibility of perforation and death [1].

Very limited information has been published on the burden of CDI to the diagnostic laboratory, and the relative incidence of disease in different clinical specialties is not well known. The present study aimed to use locally collected data from 2003 to 2007 to assess the laboratory workload associated with *C. difficile* testing and to analyse the maximal potential rates of CDI across specialties and age groups, in an area that had not been affected by the hypervirulent 027/BI/NAP 1 strain during the period of the study.

Materials and Methods

Setting

Lothian University Hospitals Division provides for approximately 620 000 people in the Edinburgh area of Southeast Scotland. The six major Edinburgh hospitals (three tertiary and three secondary care; totalling approximately 2300 beds) were included in our analysis.

Laboratory diagnosis of CDI

All faecal samples were processed in a single microbiology laboratory, in accordance with local guidelines until September

2006 and thereafter national guidelines [2]. All diarrhoeal (semi, unformed or liquid) specimens were tested from 2003 to 2008 inclusive if they were from hospital in-patients aged over 1 year old, if a diagnosis of antibiotic-associated diarrhoea or pseudomembranous colitis was present, on clinical request, or if they had been on recent antibiotic therapy. Diarrhoeal samples were tested for *C. difficile* toxins A and B by enzyme immunoassay (Techlab *C. difficile* Tox A/B II kit; TechLab, Blackburg, VA, USA) in accordance with the manufacturer's guidelines.

Inclusion criteria

All in-patients from whom an appropriate faecal sample was submitted for toxin detection were included. Patients were identified using the laboratory computer filing system (iLab, iSOFT, Banbury, UK), which stores details of every sample tested. The data included patient demographics, ward and specialty where admitted at the time of sample collection, date of sample collection, test performed and results.

A new potential CDI episode was defined in line with Scottish guidelines: 'only persons that have not been diagnosed with *Clostridium difficile*-associated diarrhoea within the previous 28 days are counted as new cases' [3]. Therefore, any repeat samples taken within 28 days after a positive result for any individual patient were excluded.

Potential CDI rates were calculated as potential episodes per 1000 in-patient occupied bed days (OBD) using data, obtained from the Information Analysis Department, Health Intelligence Unit, NHS Lothian, on annual hospital occupied bed days per speciality. Data for renal medicine and transplant surgery had to be combined as a result of the combination ward set-up.

For all analyses, other than requisition of faecal samples and the resultant workload relating to individual age groups, individuals aged less <18 years were excluded because paediatric age groups have been reported to have disproportionately high carriage rates [4,5]. This enabled a comparison with other studies, which have only included adults. With recognition that every faecal sample positive for *C. difficile* toxin does not always equate to symptomatic CDI, we have referred to potential CDI.

Costs data

The most recent published data were used to calculate potential associated costs [6].

Statistical analysis

Analysis was performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and Minitab, version 5.1.0.0 (Minitab Inc., State College, PA, USA).

TABLE 1. Incidence and number of *Clostridium difficile* infection (CDI) episodes from 2003 to 2007

Year	Total number of CDI episodes[a]	Incidence of CDI episodes per 1000 OBD[b]
2003	897	1.93
2004	875	1.51
2005	991	1.65
2006	1231	1.98
2007	928	1.48

[a]Total number of episodes of CDI per year (as defined by a stool sample positive for *C. difficile* toxin). In the case of multiple positive samples from the same patient within a 28-day period, only one was included in the total.
[b]Changes in the incidence of CDI episodes per 1000 occupied bed days (OBD).

Results

A total of 45 412 samples were tested for toxin and 6286 (13.8%) were positive. After excluding repeat positive samples from the same patient in a 28-day period, 4922 new potential episodes of CDI were identified, increasing from 875 potential cases in 2004–1231 in 2006 (Table 1). The overall rate of potential CDI for in-patients admitted from 2003 to 2007 was 1.70 cases/1000 in-patient OBD with a peak of 1.98 potential cases/1000 OBD in 2006 (Table 1). The potential number and rate of CDI fell in 2007–928 cases and 1.48 cases/1000 OBD, respectively.

Laboratory workload

The number of samples tested increased from 6493 in 2003 to 14207 in 2006, falling to 10359 in 2007. The proportion of these samples identified as positive varied annually from 11% to 16% (Fig. 1a). There was an almost equal proportion of samples from Medicine of the Elderly, all medical specialties and all surgical specialties. The former was the specialty that sent the maximum number overall, and was almost double the proportion of samples sent by the next three specialties individually (General Surgery, Gastrointestinal Medicine and Infectious Diseases). Medicine of the Elderly had the largest proportion of positive samples (22.9%) followed by Urology (17.7%), Renal Medicine/Transplant Surgery (16.8%), Respiratory Medicine (15.3%) and General Surgery (14.2%) (Fig. 1b).

Stratifying the data according to age, the largest proportion of samples was sent from the 61–80-year-old age group and the highest positivity rate was seen in those aged over 80 years. One third of all samples were sent from those aged 60 years or younger and 18.9% of positive samples came from this age group (Table 2).

Clinical workload

Renal Medicine/Transplant Surgery with 7.24 cases/1000 OBD and Intensive Care and High Dependency

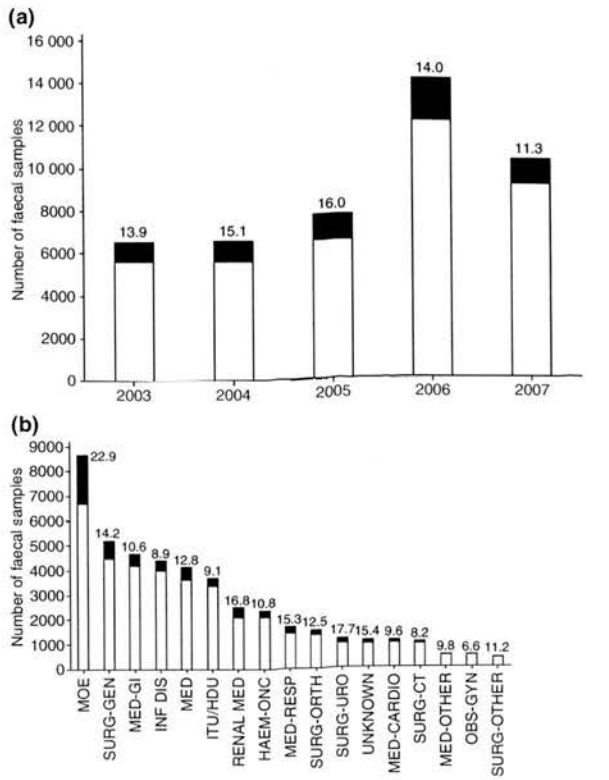

FIG. 1. Trends in number of laboratory requests for *Clostridium difficile* toxin detection 2003–2007. (a) Total number of laboratory requests for *C. difficile* toxin detection per year. The figures above the individual bars represent the percentage of positive samples per year. (b) Total number of laboratory requests for *C. difficile* toxin detection distributed by specialty. Shaded areas: positive results for *C. difficile* toxin; nonshaded areas: negative results for *C. difficile* toxin. The figures above the individual bars represent the percentage of samples that were positive per specialty. HAEM-ONC, Haematology and Oncology; INF-DIS, Infectious Diseases, ITU/HDU, Intensive Care and High Dependency Units; MED, General Medicine; MED-CARDIO, Cardiology; MOE, Medicine of the Elderly; MED-GI, Gastrointestinal Medicine; MED-OTHER, includes all other medical specialties including dermatology and rheumatology; MED-RESP, Respiratory Medicine; OBS-GYN, Obstetrics and Gynaecology; RENAL MED, Renal Medicine and Transplant Surgery; SURG-CT, Cardiothoracic Surgery; SURG-GEN, General Surgery; SURG-ORTH, Trauma and Orthopaedic Surgery; SURG-OTHER, includes all other surgical specialties including vascular; SURG-URO, Urology; UNKNOWN, Samples that could not be allocated to a particular specialty.

(ITU + HDU) with 6.23 cases/1000 OBD had the highest potential CDI incidences followed by Infectious Diseases and Gastrointestinal Medicine, where rates were 5.50 and 4.42 cases/1000 OBD, respectively. By comparison, Medicine of the Elderly had an incidence of 1.69 cases/1000 OBD. In addition General Surgery and Urology both had higher rates than Medicine of the Elderly (Fig. 2a).

TABLE 2. Number of samples tested (total and proportion positive) by the laboratory, distributed according to age group

Age range	Number of samples (%) Tested	Positive	Percent positivity per age group (%)
0–20	1973 (4.3)	94 (1.5)	4.8
21–40	4588 (10.1)	293 (4.7)	6.4
41–60	8697 (19.2)	800 (12.7)	9.2
61–80	18211 (40.1)	2772 (44.1)	15.2
>80	11943 (26.3)	2327 (37.0)	19.5
Total	45412 (100)	6286 (100)	5.9

On review of the trends in potential CDI incidence in the seven specialties with the highest rates over the 5-year period (Fig. 2b), all latterly had a reduction in CDI cases despite following varying trends. ITU + HDU, Infectious Diseases and Gastrointestinal Medicine appeared to have the greatest reduction. For each specialty, the rates peaked at different times. Renal Medicine/Transplant Surgery had a maximal rate in 2006, ITU + HDU in 2004, Infectious Diseases and Gastrointestinal Medicine in 2005, Urology in 2006, General Surgery in 2003 and Medicine of the Elderly in 2006.

The incidence of potential CDI rates also increased with age from 0.45 cases/1000 OBD in the 0–20-year-old age group to 2.02 cases/1000 OBD in the 61–80-year-old age group. Similar to the specialty trends, there was latterly a reduction in all age groups. Over the period for those aged above 41 years, each group appeared to follow a similar trend, with peaks in incidences in 2003 and 2006. However, in the younger two age groups, the peak occurred later: in 2005 in the 21–40-year-old age group and in 2006 in the 0–20-year-old age group (Fig. 3).

Of all toxin-positive patients, 12% were transferred through a minimum of two specialties and 17% of these through a minimum of three to a maximum of six specialties. These transfers also included movement through acute-service areas: Accident and Emergency, Combined Assessment Units and Acute Receiving Units.

Discussion

The *C. difficile*-related burden handled by the laboratory and the potential clinical workload over a 5-year period within Edinburgh were assessed. The results represent the first study from Scotland, and possibly the first ever, demonstrating the CDI laboratory workload. The recent CDI trends

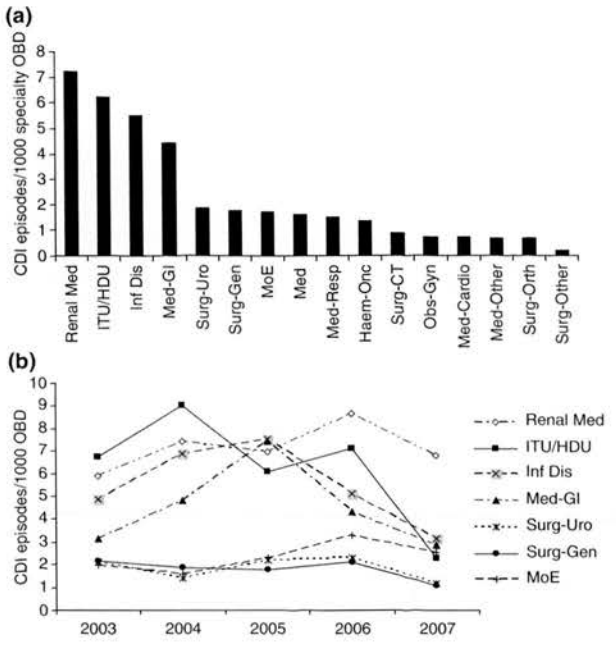

FIG. 2. Distribution of *Clostridium difficile* infection (CDI) incidence among the various specialties from 2003 to 2007. (a) Annual incidence of CDI in the seven specialties with the highest overall incidence of CDI [episodes per 1000 occupied bed days (OBD)]. (b) The overall incidence of CDI episodes per 1000 OBD per specialty. HAEM-ONC, Haematology and Oncology; INF-DIS, Infectious Diseases, ITU/HDU, Intensive Care and High Dependency Units; MED, General Medicine; MED-CARDIO, Cardiology; MOE, Medicine of the Elderly; MED-GI, Gastrointestinal Medicine; MED-OTHER, includes all other medical specialties including dermatology and rheumatology; MED-RESP, Respiratory Medicine; OBS-GYN, Obstetrics and Gynaecology; RENAL MED, Renal Medicine and Transplant Surgery; SURG-CT, Cardiothoracic Surgery; SURG-GEN, General Surgery; SURG-ORTH, Trauma and Orthopaedic Surgery; SURG-OTHER, includes all other surgical specialties including vascular, SURG-URO, Urology.

and maximal potential specialty-dependent rates are also reported. Although the study reviews presumptive rates of specialty-based CDI based on toxin-positive samples rather than symptomatic disease, it provides valuable information on the burden of disease prior to mandatory data reporting, introduced in September 2006, and for age groups below 65 years.

A fall in the rate of CDI occurred between to 2003–2004 and 2006–2007. The initial reduction may be secondary to increased awareness and improved infection control practices with the subsequent rise occurring if infection control measures relaxed with an associated increase in antibiotic usage (e.g. of fluoroquinolones). The decline seen latterly may represent enhanced infection control measures including

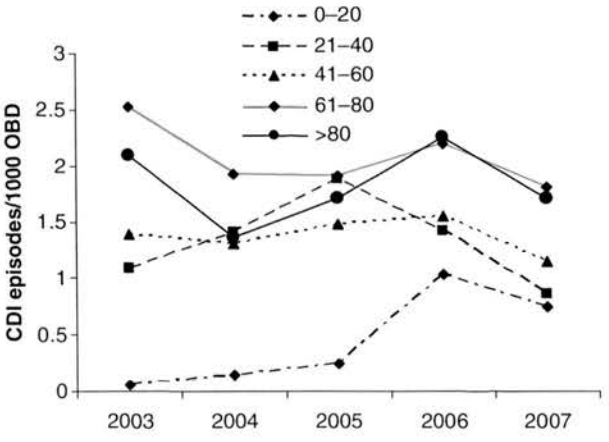

FIG. 3. Distribution of annual age-related *Clostridium difficile* infection (CDI) incidence [episodes per 1000 occupied bed days (OBD)] from 2003 to 2007.

improvements in CDI-related education and judicious antibiotic use.

This is converse to some other reported figures. In Canada, an increase in incidence from 2.5 cases/1000 admissions to 4.6 cases/1000 admissions occurred from 2002 to 2007 [7]. In Spain, where ribotype 027 is not prevalent, the prevalence rates have increased from 0.39 to 1.22 cases/1000 hospitalized patients from 1999 to 2007 [8]. The incidence of 1.27 CDI cases/1000 OBD in the whole of Scotland from October 2006 to September 2007 for those aged 65 years and over [4] was lower than the 1.99 CDI cases/1000 OBD, for the same period and cohort of patients, in the present study. This may be a result of our study representing potential CDI in a concentrated urban population.

There was a 54% increase in the number of samples analysed by the laboratory from 2003 to 2006. Although the number decreased from 2006 to 2007, there was a 37% increase from 2003 to 2007. This will have impacted upon staff as well as upon an appropriate analysis of samples, timely data reporting, storage of samples and further testing of repeat samples from previously positive patients.

This increase will also have had implications for ward-based staff with regard to rapid and appropriate patient isolation, cleaning procedures and continued patient management.

One-third of samples were from those aged 60 years and under, and this population also accounted for approximately one-fifth of the total positives (Table 2).

This was similar to results from the recent 2009 European Study (M. Bauer, unpublished data) where 37% of samples were submitted from those aged 65 years or under. In the Netherlands in 2005, 42% of samples were submitted from this age group [9]. In the present study, CDI toxin positivity was identified in all age groups, including that aged

0–20 years, and the potential CDI incidence in those aged 21–40 and 41–60 years was not largely dissimilar from that in those aged over 60 years. It should be noted that the potential false positive rates will be higher among the younger age groups because the potential prevalence in these age groups (aged 60 years and under) is <10%. With mandatory data reporting in Scotland initially introduced for those aged 65 years and above only, a large and important subset of patients was excluded from national figures. Subsequent to May 2009, mandatory data reporting in Scotland will now include those aged 16 years and above.

The elderly population is deemed to be at high risk for CDI; however, it is no longer the only at-risk group [10]. An analysis of our data by specialties revealed that Obstetrics and Gynaecology patients, a group with minimal CDI, have a low but noteworthy incidence of disease. Because there are only a few reports of peripartum CDI in young women [11], they, along with paediatric patients, comprise a sub-group where there should be vigilance and a high degree of clinical suspicion in symptomatic patients.

Advanced age has been found to be an independent predictor of mortality in patients with CDI [12]. However, in the present study, Medicine of the Elderly had a much lower incidence than several other specialties, although it accounted for the largest number of samples tested and had the greatest proportion of positive samples. Renal Medicine/Transplant Surgery, ITU, Infectious Diseases, Gastrointestinal Medicine, Urology and General Surgery all had a higher incidence of CDI.

This is similar to a study from Amsterdam where CDI occurred more frequently in ITU patients and those admitted to surgery [13]. Conversely, in Spain, admission to a general medical ward was associated with higher CDI prevalence as opposed to ITU admission [8].

Renal Medicine/Transplant Surgery had the highest incidence of CDI over the study period. This may reflect overall antibiotic usage, immunosuppression and repeated admissions. Chronic haemodialysis patients have a greater risk of developing nosocomial infections including CDI [14] and renal insufficiency is associated with increased risk of CDI after live donor liver transplantation [15]. Liver disease is also independently associated with severe CDI outcome [7].

The recognition of patients with severe CDI, subsequent to outbreaks in Canada [16] and Stoke Mandeville (Bucks, UK) [17], could have resulted in an increased number of patients with CDI being referred to ITU and hence the observed peak in 2004. The marked decline in potential CDI incidence observed afterwards within ITU and HDU areas may be a result of increased awareness, stringent infection control and the overall decrease seen across all specialties. In the present study, the incidence of CDI among ITU + HDU patients was 6.23 cases/1000 OBD.

Lawrence *et al.* [18] reported an incidence of CDI in ITU to be 3.2 cases/1000 patient days in the absence of an outbreak. This may be a result of differences in patient populations and our figure represents a maximal potential incidence, which includes patients transferred to ITU with CDI. In 2008, a study reported that a specific subgroup of young critically ill trauma patients admitted to ITU developed CDI even though administration of antibiotics was for surgical prophylaxis only [19].

Gastrointestinal Medicine may handle a substantial burden of CDI as a result of a high prevalence among inflammatory bowel disease patients. CDI has been reported to be eight-fold more prevalent in patients with ulcerative colitis and five-fold more prevalent in patients with Crohn's disease compared to the general population [20,21].

General Surgery had a plateau from 2003 to 2006 despite a rise in the total CDI incidence in 2006. This contrasts to other studies that noted a rise in CDI among patients admitted in surgery, particularly for patients requiring surgical intervention for CDI [22–24]. Although patients are at risk of acquiring CDI within the specialty itself, patients are also referred for surgical intervention due to severe fulminant *C. difficile* colitis and typhlitis. This may suggest that patients were not being referred for surgical review; disease severity did not warrant surgery or patients were not suitable for it. Early surgical referral should always be made for severe fulminant colitis as a total colectomy prior to acute respiratory or renal failure in those aged over 65 years is associated with decreased mortality [22].

The incidence of CDI in Urology did not vary greatly and the overall incidence was 1.84 cases/1000 OBD. This was higher than observed by Hossain *et al.* [25] who reported an incidence of 0.66 cases/1000 OBD.

Approximately one-tenth of all toxin-positive patients were transferred through a minimum of two specialties when remaining toxin positive. The maximum number of inter-specialty transfers (and therefore wards) for an individual patient was six. This does not take into account the number of individual bed moves within the same ward. *C. difficile* spores contaminate the hospital environment and are more likely to be recovered from a room housing a CDI patient [26] and this can persist for up 40 days after discharge [27,28]. Acquisition of CDI is more rapid, occurring after 3.2 days for patients sharing a room with a CDI patient compared to 18.9 days for those who do not have close contact [29].

Estimated costs for toxin testing alone were approximately £126 500 (€143 000). On the basis of the most

recent published European costs of CDI by Vonberg *et al.* [6], the excess costs per in-patient case was €7147. This agrees with the only published figures available for Great Britain from Wilcox *et al.* [30] in 1996, where extra costs were estimated at £4000 (€4500) per case. Without adjusting for inflation, the potential excess costs for CDI patients over the study period was £20 000 000 (€22 500 000).

Most local strains of *C. difficile* associated with CDI belong to ribotypes 001 and 106 [31]. Ribotype 027, and now 078 [32], is associated with increasing CDI rates and more severe disease.

The incidence of CDI appears similar to regions where 027 was prevalent. Control of CDI should not be focused solely on 027 and strategies to reduce CDI should consider the pathogenic potential of local strains. However, the absence of hypervirulent types may explain the plateau in the incidence observed in General Surgery.

The present study has confirmed that CDI incidence increases with age but varies among specialties, indicating where an assessment of the risk of developing CDI should be targeted.

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Transparency Declaration

None declared.

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Potential Differences in the Use of Abdominal CT and Imaging Findings between Differing *Clostridium difficile* Strains.

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Severe *Clostridium difficile* infection (CDI) is increasingly being assessed by computed tomography (CT). Our aim is to assess imaging findings in patients affected by different *C. difficile* strains.

All *C. difficile* positive faecal samples from 1 August 2010 to 31 July 2011 were cultured and PCR ribotyped. A retrospective review of prospectively collected clinical and radiological data was then performed on all positive, symptomatic, hospitalised adult patients.

453 CDI patient episodes were identified in 350 symptomatic patients. Overall 72 CT-scans were performed, 55 scans demonstrated colonic involvement, with pancolitis in only 31%. Colonic changes were present in 35% of episodes prior to the availability of a *C. difficile* positive result (median 2 days).
Ribotype 001 (73 episodes) was the commonest identified *C. difficile* strain and a CT was performed in only 11%, compared with ribotype 078, the 6th commonest strain (19 episodes) where CT was performed in 52%. Patients with 078 had a greater colonic wall thicknesses (median 16mm) compared with the remaining cohort (median 11mm). Ascites was present in 64% of the scanned 078 patients compared with 31% in the remaining cohort.

CDI should be considered in the differential diagnosis of colitides, even prior to a *C.difficile* positive result. Increased colonic wall thickness and ascites were more common in the 078 episode group with a greater proportion of CT scans performed. 078 appears to stimulate a greater inflammatory response within the colon, which has not been previously described.

CT imaging can provide valuable biological data in assessing potential actions between different strains of Clostridium difficile. In particular emerging strains in which pathological mechanisms have not been established.

Although the hypervirulent 027 strain is not endemic to our area; another potentially emerging aggressive strain-078 has become prevalent.

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Environmental Contamination Of *Clostridium Difficile* In A Radiology Ultrasound Department.

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Aims: To assess the presence of *Clostridium difficile* and the potential risk of transmission to patients and health-care professionals in a high-risk clinical area within a radiology department.
Methods: Contact plates (cycloserine, cefoxitin, egg-yolk: CCEY) were used to sample 35 environmental sites for *C. difficile*. These were designated as high or moderate risk within two ultrasound (US) rooms with the greatest in-patient movement. Blood agar contact plates were also used to calculate overall colony counts in order to assess areas of greatest contamination. Each site was sampled at the start and end of a standard 8 hour working day. Contact plates were then incubated and evaluated according to standard or manufacturer protocols. The blood agar contact plates were divided for both aerobic and anaerobic incubation. This gave a total of 70 CCEY and 140 blood agar plates.
Results: Two of 70 CCEY contact plates were culture positive for *Clostridium difficile*. These were isolated from the curtain rail and under-surface of the patient couch frame belonging to the same cubicle. Both samples were positive for toxins A/B, via enzyme immunoassay, and sensitive to both metronidazole and vancomycin via E-test. The highest overall combined aerobe and anaerobe colony counts were obtained from the patient couches, computer keyboards, floor, US probe holder, US control panel and US gel warmer with a median colony count of 198 (range 123 to 954). The areas demonstrating the greatest increase in colony counts over the course of the day were the US control panel, the US gel warmer and the floor.
Conclusions: Overall minimal *C. difficile* contamination was identified. The study however identifies areas which may be overlooked by current cleaning protocols where *C. difficile* spores can exist and act as a potential source of hospital acquired infections. In addition potential areas of increased contamination have been highlighted, allowing greater awareness of areas to target during standard decontamination procedures.

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Identification of *Clostridium difficile* in Colorectal Surgery.

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Objectives: *Clostridium difficile* Infection (CDI) has become a growing concern world-wide with an increased reported incidence among patients admitted to surgery. Our aim was to prospectively review the role of toxigenic culture in the diagnosis of CDI in colorectal surgical in-patients from December 2007 to January 2009.

Methods: All faecal samples, submitted to Lothian University Hospitals Division, were processed in a single enteric laboratory following national guidelines – all hospital diarrhoeal in-patient samples from those aged 1year and above were tested for *C. difficile* toxins A and B by enzyme immunoassay. All faecal samples submitted to the laboratory from colorectal surgical in-patients were reclaimed for toxigenic culture (culture on selective media and EIA).

Results: 632 samples, from 483 adult patients (median age 71 years, ranging from 18-100 years) were reclaimed for toxigenic culture. Of these 105 samples (16.6%) were found by the laboratory to be positive by EIA.
Following toxigenic culture a further 72 samples (11.4%) were identified as positive. 38 patients, who were symptomatic at the time of testing, were not identified with CDI during their admission.
5 patients whose samples were not tested as they were not diarrhoeal ($\leq$ 4 on the Bristol stool chart) were toxigenic culture positive. These patients were symptomatic of CDI and subsequent diagnosis was delayed in 3 of these patients and not diagnosed in 2 patients.
Seven samples found to be toxin positive by the lab were culture negative.

Conclusions: CDI diagnosis or recognition at present may be delayed, as with current national guidelines CDI detection is based solely upon *C. difficile* toxins A+B EIA. Current resources cannot support toxigenic culture for all suspected samples and this has implications in regards to delayed treatment, further patient management and infection control procedures. With current testing including diarrhoeal samples only, a proportion of patients with CDI may go undiagnosed or have diagnosis delayed.

Presented at Anaerobe; Philadelphia, 2010.

Clostridium difficile in the Hospital Environment.

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Objectives: *Clostridium difficile* Infection (CDI) has become a growing concern world-wide with an increased reported incidence among patients admitted to surgery. Our aim was to assess the presence of *Clostridium difficile* spores within the surgical wards of a large tertiary referral hospital, to assess the common areas contaminated and the effects of introducing new cleaning procedures, from December 2007 to January 2009.

Methods: 4 acute surgical wards were included and 180 CCEY (cefoxitin, ceftriaxone and egg yolk) contact plates were used every 4-6 weeks to sample the environment. The same areas were sampled on each occasion in the same manner over a 14 month period. At the 7 month time point chlorine based cleaning agent was introduced in addition to the original non-chlorine based detergent. The plates were incubated for 5 days and all *Clostridium difficile* colonies were tested for toxins A+B.

Results: Over the study period 5% of all contact plates were positive for toxigenic *Clostridium difficile* colonies. One ward had 80% fewer positive plates than the other wards. This is likely to represent the differing patient cohort within the ward (overnight stay/daycase patients).
Contaminated areas included bed frames, patient tables, bedside lockers, door handles, sinks, bathroom floors and electronic sphygmomanometers. Bed frames produced the greatest number of positive contact plates when compared with the other surfaces tested. Only 11% of the positive contact plates were from rooms which were occupied by a patient with CDI at the time of sampling.
There was an initial 86% reduction in the number of positive contact plates following the introduction of chlorine based cleaning agents during months 7-8. Following this initial reduction, a plateau of between 48-62% reductions was maintained for the remaining months when compared to the first 6month period.

Conclusions: The environment is an important reservoir for Clostridium difficile spore contamination and hence potential patient development of CDI. Attention to the cleaning of areas which may be overlooked such as electronic sphygmomanometers should be paid. The use of chlorine based cleaning agents and meticulous cleaning procedures are vital components of infection control procedures and the control of CDI dissemination.

Presented at Anaerobe; Philadelphia, 2010.

Clostridium difficile: changing epidemiology trends 2000-2007

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Objectives: *Clostridium difficile* Infection (CDI) has become a growing concern world-wide with an increased reported incidence and an increase in the associated financial burden. Our aim therefore was to review trends in CDI occurring from 2000-2007 inclusive.

Methods: All patients admitted to Lothian University Hospitals Division (LUHD) tested for *C. difficile* toxins A+B by EIA were included. Retrospective analysis of prospectively collected data was performed. The number of occupied bed days was provided by NHS-Lothian Statistics Department. The most recent published costs associated with CDI were used to estimate potential costs to Lothian NHS Trust.

Results: 50590 faecal samples were tested for *C. difficile* toxins from 2000-2007 inclusive; of these 7301 samples were positive. Overall CDI was identified in 15.2 cases/10000 patient days and 5.8 cases/1000 in-patient hospital admissions.

The incidence of identified CDI rose from 3.6cases/10000 patient days in 2000 to 14.8cases/10000 patient days in 2007. Incidence also increased with age from 3.3cases/10000 patient days in the 0-20 years age group to 18.1cases/10000 patient days in the 61-80 years age group.

Renal Medicine and Intensive Care had the highest incidences of identified CDI with greater than 57cases/10000 patient days each followed by Infectious Diseases and Gastrointestinal Medicine whose rates were 47.5 and 42.6 cases/10000 patient days respectively . Medicine of the Elderly in comparison had an incidence of 19.5cases/10000 patient days.

Of note 10% of all patients were transferred through a minimum of two specialties during the period in which they remained positive for *C. difficile* toxins.

Estimated costs over the study period for toxin testing alone were in the region of £126,500 and the minimal potential hospitalisation costs of patients with CDI was in the region of £20,000,000.

Conclusion: The incidence of patients identified with CDI has risen markedly and not surprisingly the incidence has also been noted to increase with age. Medicine of the Elderly however had a much lower incidence than several other specialties and therefore risk assessment of CDI development and containment should now also be targeted within other specialties.

With 10% of identified CDI patients transferred through different specialties and the significant financial burden CDI imposes on healthcare institutions judicious application of infection control measures remains an important factor to prevent CDI spread.

Presented at the European Congress of Clinical Microbiology and Infectious Diseases; Helsinki, 2009.

The Incidence of *Clostridium difficile* in Colorectal Surgery

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Purpose : There has been increased interest in hospital-acquired infections, in particular Clostridium difficile (C. difficile) recently. Our aim was to review the incidence of C. difficile within the adult in-patient population presenting to a University Hospitals Trust and the impact upon the Colorectal Surgical Service over a 7-year period (2000-2006 inclusive).

Methods : Cross-referencing Medical Microbiology, Pathology and Surgical Audit databases identified in-patients, from the four main hospitals within the Trust, diagnosed with C.difficile. Retrospective analysis of prospectively collected outcome data was then performed.

Results : 41,356 faecal samples were tested over the 7-year period. 15% (6325) of faecal samples tested were toxin positive, via ELISA, for C. difficile. 3895 patients were diagnosed with Clostridium difficile associated disease (CDAD). 7.6% (296) of patients diagnosed with CDAD were treated by the Colorectal Surgical Unit only 23 patients underwent surgery. A detailed analysis is demonstrated below.

Conclusions : Over the 7-year study period, there has been an exponential increase in the number of faecal samples and consequently the number of patients tested for and diagnosed with CDAD. Despite the proportion of colorectal patients, compared with the total number of patients, treated for CDAD each year not appreciably increasing. The number of individual in-patients treated conservatively for CDAD by the Colorectal Surgical Service has markedly increased impacting on the overall burden of the Colorectal Surgical Unit.

Year (12 months analysis period)	Number of Faecal Samples Tested	Number of C. difficile positive samples	Number of C. difficile positive patients	Number of C. difficile positive Colorectal patients	Number of patients who underwent surgery.
2000	532	150	98	4	1
2001	1185	273	175	26	5
2002	3767	653	463	35	0
2003	6833	980	612	45	2
2004	6600	998	687	49	5
2005	7841	1237	806	53	5
2006	14598	2034	1054	84	5

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Clostridium difficile in the paediatric population.

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Objectives: Clostridium difficile Associated Disease (CDAD) cases are increasing. There has been a shift in trends with a rising number of cases being reported in low-risk populations' e.g. children and young peri-partum women. Our aim was to review the prevalence of C. difficile in the in-patient paediatric population presenting to a Children's Hospital within a University Hospitals Trust from 2000-2006 inclusive, and to further assess the data set for 2006.

Methods: Patients were identified via Medical Microbiology databases. Patients ranging from greater than one-month to less than 14 years were included. Retrospective analysis of prospectively collected outcome data was then performed.

Results: There has been an increase in the number of diagnosed paediatric CDAD cases over the last year. A detailed breakdown is shown below.

Age Range (years)	2000	2001	2002	2003	2004	2005	2006
>1 month - <1	0	0	0	0	0	0	3
1 - 4	0	0	0	0	1	2	12
5 - 13	0	1	2	1	1	2	7

Further analysis of data for 2006 revealed 603 faecal samples were tested for C. difficile of which 33 samples were positive. Taking into account multiple samples testing, for individual patients, 22 patients were diagnosed with CDAD out of 335. Of the 22 positive patients there was no significant gender bias (Male:Female=10:12) and the median age was 3years (range 2months - 13years). For this cohort 117 samples were analysed ranging from 1-31 samples per patient over a time period of 1-331days. Only 54% of patients were found to be toxin positive on their first sample. A median period of 23days (range 2 – 102 days) was conceded prior to detection of C. difficile toxin, from the time of their first negative sample being assessed to the ultimate positive sample, a median of 3 (range 2-6) samples was sent prior to detection. 23% of positive patients had CDAD re-diagnosed on subsequent samples following a negative sample. In these cases the median duration was 22 days (range 15-104days) following a median of a further 2 samples being analysed (range 2-4).

Conclusions: The diagnosis of C. difficile is increasing in this previously low-risk paediatric population and therefore is a clinical diagnosis that must be considered early. Culture in conjunction with toxin testing may have led to an earlier diagnosis in nearly 50% of patients and there is a possibility that re-infection/re-colonisation may occur in up to a quarter of Paediatric cases. Mandatory data reporting is only required for patients over 65years resulting in this important sub-set being excluded.

Presented at the European Congress of Clinical Microbiology and Infectious Diseases; Barcelona, 2008.